

**DITERPENOID COMPOUNDS, COMPOSITIONS THEREOF
AND THEIR USE AS ANTI-CANCER OR ANTI-FUNGAL AGENTS**

This application claims the benefit of U.S. provisional application Serial No. 60/431,096, filed December 5, 2002, the entire disclosure of which is incorporated by reference herein in its entirety.

1. FIELD OF THE INVENTION

The present invention relates to Diterpenoid Compounds, compositions comprising an effective amount of a Diterpenoid Compound, and methods useful for treating or preventing cancer or a neoplastic disorder comprising administering an effective amount of a Diterpenoid Compound. The compounds, compositions, and methods of the invention are also useful for inhibiting the growth of a cancer cell or neoplastic cell, or for inducing apoptosis in a cancer or neoplastic cell. The compounds, compositions, and methods of the invention are further useful for treating or preventing a fungal infection. The compounds, compositions, and methods of the invention are also useful for inhibiting the growth of a fungus.

2. BACKGROUND OF THE INVENTION

2.1 CANCER AND NEOPLASTIC DISEASE

Cancer affects approximately 20 million adults and children worldwide, and this year, more than 9 million new cases will be diagnosed (International Agency for Research on Cancer; www.iarc.fr). According to the American Cancer Society, about 563,100 Americans are expected to die of cancer this year, more than 1500 people a day. Since 1990, in the United States alone, nearly five million lives have been lost to cancer, and approximately 12 million new cases have been diagnosed.

Currently, cancer therapy involves surgery, chemotherapy and/or radiation treatment or eradicate neoplastic cells in a patient (see, for example, Stockdale, 1998, "Principles of Cancer Patient Management", in Scientific American: Medicine, vol. 3, Rubenstein and

Federman, eds., Chapter 12, Section IV). All of these approaches pose significant drawbacks for the patient. Surgery, for example, can be contraindicated due to the health of the patient or can be unacceptable to the patient. Additionally, surgery might not completely remove the neoplastic tissue. Radiation therapy is effective only when the irradiated neoplastic tissue exhibits a higher sensitivity to radiation than normal tissue, and radiation therapy can also often elicit serious side effects. (*Id.*) With respect to chemotherapy, there are a variety of chemotherapeutic agents available for treatment of neoplastic disease. However, despite the availability of a variety of chemotherapeutic agents, traditional chemotherapy has many drawbacks (see, for example, Stockdale, 1998, "Principles Of Cancer Patient Management" in Scientific American Medicine, vol. 3, Rubenstein and Federman, eds., ch. 12, sect. 10). Almost all chemotherapeutic agents are toxic, and chemotherapy can cause significant, and often dangerous, side effects, including severe nausea, bone marrow depression, immunosuppression, etc. Additionally, many tumor cells are resistant or develop resistance to chemotherapeutic agents through multi-drug resistance.

Therefore, there is a significant need in the art for novel compounds and compositions, and methods that are useful for treating cancer or neoplastic disease with reduced or without the aforementioned side effects. Further, there is a need for cancer treatments that provide cancer-cell-specific therapies with increased specificity and decreased toxicity.

2.2 FUNGAL INFECTION AND RELATED HEALTH ISSUES

Fungi are eukaryotic microorganisms and can occur as yeasts, molds, or as a combination of both forms. Some fungi are capable of causing superficial, cutaneous, subcutaneous, systemic or allergic diseases. Yeasts are microscopic fungi consisting of solitary cells that reproduce by budding. Molds, in contrast, occur in long filaments known as hyphae, which grow by apical extension.

Known fungal and mycotic pathogens include, but are not limited to, *Absidia* spp., *Actinomadura madurae*, *Actinomyces* spp., *Allescheria boydii*, *Alternaria* spp., *Anthopsis deltoidea*, *Apophysomyces elegans*, *Arnim leoporinum*, *Aspergillus* spp., *Aureobasidium pullulans*, *Basidiobolus ranarum*, *Bipolaris* spp., *Blastomyces dermatitidis*, *Candida* spp., *Cephalosporium* spp., *Chaetoconidium* spp., *Chaetomium* spp., *Cladosporium* spp., *Coccidioides immitis*, *Conidiobolus* spp., *Corynebacterium tenuis*, *Cryptococcus* spp.,

Cunninghamella bertholletiae, *Curvularia* spp., *Dactylaria* spp., *Epidermophyton* spp., *Epidermophyton floccosum*, *Exserophilum* spp., *Exophiala* spp., *Fonsecaea* spp., *Fusarium* spp., *Geotrichum* spp., *Helminthosporium* spp., *Histoplasma* spp., *Lecytophora* spp., *Madurella* spp., *Malassezia furfur*, *Microsporum* spp., *Mucor* spp., *Mycocentrospora acerina*, *Nocardia* spp., *Paracoccidioides brasiliensis*, *Penicillium* spp., *Phaeosclera dematioides*, *Phaeoannellomyces* spp., *Phialemonium obovatum*, *Phialophora* spp., *Phoma* spp., *Piedraia hortai*, *Pneumocystis carinii*, *Pythium insidiosum*, *Rhinocladiella aquaspersa*, *Rhizomucor pusillus*, *Rhizopus* spp., *Saksenaea vasiformis*, *Sarcinomyces phaeomuriformis*, *Sporothrix schenckii*, *Syncephalastrum racemosum*, *Taeniolella boppii*, *Torulopsis* spp., *Trichophyton* spp., *Trichosporon* spp., *Ulocladium chartarum*, *Wangiella dermatitidis*, and *Xylohypha* spp. Other fungi that might have pathogenic potential include, but are not limited to, *Thermomucor indicae-seudaticae*, *Radiomyces* spp., and other species of known pathogenic genera. There are also reports implicating *Saccharomyces* as a human pathogen (*e.g.*, Fungemia with *Saccharomycetacea*, H. Nielson, J. Stenderup, & B. Bruun, Scand. J. Infect. Dis. 22:581-584, 1990). In recent years, there has been a marked increase in the number of serious mycoses as a result of the growing number of immunosuppressed and immunocompromised individuals, such as transplant recipients, patients receiving chemotherapy, and HIV-infected individuals, and thus greater attention has been devoted to the need to develop safer and more effective antifungal agents.

Fungal infection is also a significant problem in veterinary medicine and in agriculture. Products that are susceptible to fungal infestation include wood products, textiles, plastics, paper, rubber, adhesives, emulsion polymers, leather, cosmetics, household disinfectants, deodorants, and paint (C.C. Yeager, Fungicides in Industry, in *Antifungal Compounds*, M. Siegel and H. Sisler, eds., Marcel Dekker Inc., NY, 1977).

2.2.1 CURRENT THERAPIES

The mechanism of action of four main classes of anti-fungal agents is summarized below:

Polyene Antifungal Drugs

Amphotericin, nystatin, and pimarinic interact with sterols in the cell membrane (ergosterol in fungi, cholesterol in humans) to form channels through which small molecules leak from the inside of the fungal cell to the outside.

Azole Antifungal Drugs

Fluconazole, itraconazole, and ketoconazole inhibit cytochrome P450-dependent enzymes (particularly C14-demethylase) involved in the biosynthesis of ergosterol, which is required for fungal cell membrane structure and function.

Allylamine and Morpholine Antifungal drugs

Allylamines (naftifine, terbinafine) inhibit ergosterol biosynthesis at the level of squalene epoxidase. The morpholine drug amorolfine inhibits the same pathway at a later step.

Antimetabolite antifungal drugs

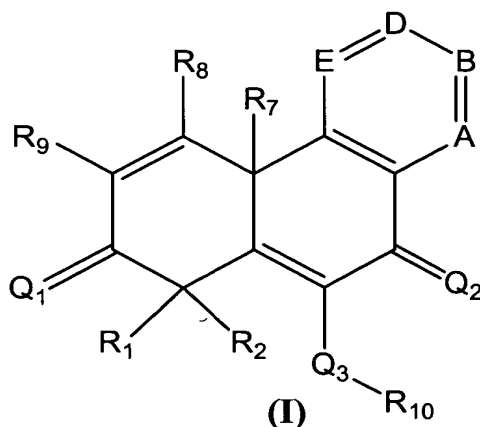
5-Fluorocytosine acts as an inhibitor of both DNA and RNA synthesis via the conversion of 5-fluorocytosine to 5-fluorouracil.

Many of the drugs currently available for treatment of mycoses have significant side effects or lack effectiveness against some important pathogens. For example, amphotericin B, an antifungal polyene macrolide antibiotic, has both short-term and long-term adverse effects, ranging from nausea and vomiting to kidney damage. Some evidence exists for the development of resistance to these drugs. There is therefore an ongoing need for novel antifungal drugs with few, if any, side effects and with effectiveness against pathogens for which current drugs are inadequate.

Citation of any reference in Section 2 of this application is not an admission that the reference is prior art to the application.

3. SUMMARY OF THE INVENTION

The present invention encompasses compounds having the Formula (I):



and pharmaceutically acceptable salts thereof, wherein:

Q_1 and Q_2 are independently =O, =S, =NH or =N-NHR, where R is -H, -C₁-C₁₀ alkyl, or -aryl;

Q_3 is -O-, -S-, or -N(H)-;

R_1 and R_2 are independently -H, -halogen, -amino, -C₁-C₁₀ alkyl, -C₁-C₁₀ alkoxy, -C₁-C₁₀ (hydroxy)alkyl, -C₁-C₁₀ (amino)alkyl, -C₁-C₁₀ (halo)alkyl, -C₂-C₁₀ alkenyl, -C₂-C₁₀ alkynyl, (C₃-C₇) cycloalkyl, -aryl, C₁-C₁₀ (aryl)alkyl, or three- to seven-membered non-aromatic heterocycle, or R_1 , R_2 and the carbon atom to which they are both attached are taken together to form a (C₃-C₇) cycloalkyl group or a three- to seven-membered non-aromatic heterocycle;

A is N or CR₃; B is N or CR₄; D is N or CR₅; E is N or CR₆, at least one of A, B, D and E being CR₃, CR₄, CR₅ or CR₆, respectively;

each R_3 , R_4 , R_5 and R_6 is independently -H, -halogen, -CN, -NH₂, -NO₂, -COOH, -C(O)NH₂, -SH, -S(O)NH₂, -S(O)₂NH₂, -C₁-C₁₀ (oxy)alkyl, -C₁-C₁₀ alkyl, -C₁-C₁₀ alkoxy, -C₁-C₁₀ (hydroxy)alkyl, -C₁-C₁₀ (amino)alkyl, -C₁-C₁₀ (halo)alkyl, -C₂-C₁₀ alkenyl, -C₂-C₁₀ alkynyl, -(C₃-C₇) cycloalkyl, -aryl, -C₁-C₁₀ (aryl)alkyl, three- to seven-membered non-aromatic heterocycle, five- to seven-membered aromatic heterocycle, -CH₂OR₁₁, -OCH₂OR₁₁, -OC(O)R₁₁, -C(O)R₁₁, -OC(O)OR₁₁, -OC(O)NR₁₁, -C(O)OR₁₁, -C(O)NR₁₁, -OP(O)(OR₁₁)₂, -SR₁₁, -S(O)₂NHR₁₁, -SOR₁₁, -S(O)₂R₁₁, -NHC(O)R₁₁, -NHSOR₁₁, or NHS(O)₂R₁₁; or

R_3 and R_4 and the carbon atoms to which they are attached are taken together to form a (C₃-C₇) cycloalkenyl group, a five- to seven-membered non-aromatic heterocycle, or a five- to seven-membered aromatic heterocycle; or

R₅ and R₆ and the carbon atoms to which they are attached are taken together to form a (C₃-C₇) cycloalkenyl group, a five- to seven-membered non-aromatic heterocycle, or a five- to seven-membered aromatic heterocycle; or

R₄ and R₅ and the carbon atoms to which they are attached are taken together to form a (C₃-C₇) cycloalkenyl group, a non-oxygen-containing five-membered non-aromatic heterocycle, a non-oxygen-containing five-membered aromatic heterocycle, a six- to seven-membered non-aromatic heterocycle or a six- to seven-membered aromatic heterocycle;

R₇ is -H, -C₁-C₁₀ alkyl, or -C₁-C₁₀ alkoxy;

R₈ and R₉ are each independently -H, -halogen, -CN, -NH₂, -NO₂, -COOH, -C(O)NH₂, -SH, -S(O)NH₂, -S(O)₂NH₂, -C₁-C₁₀ (oxy)alkyl, -C₁-C₁₀ alkyl, -C₁-C₁₀ alkoxy, -C₁-C₁₀ (hydroxy)alkyl, -C₁-C₁₀ (amino)alkyl, -C₁-C₁₀ (halo)alkyl, -C₂-C₁₀ alkenyl, -C₂-C₁₀ alkynyl, -(C₃-C₇) cycloalkyl, -aryl, -C₁-C₁₀ (aryl)alkyl, three- to seven-membered non-aromatic heterocycle, five- to seven-membered aromatic heterocycle, -CH₂OR₁₁, -OCR₁₁, -OC(O)R₁₁, -C(O)R₁₁, -OC(O)OR₁₁, -OC(O)NR₁₁, -C(O)OR₁₁, -C(O)NR₁₁, -OP(O)(OR₁₁)₂, -SR₁₁, -SOR₁₁, -S(O)₂R₁₁, -S(O)₂NHR₁₁, -NHSR₁₁, -NHSOR₁₁, or -NHS(O)₂R₁₁;

R₁₀ is -H, -C₁-C₁₀ alkyl, -C₃-C₇ cycloalkyl, -C(O)C₁-C₁₀ alkyl, -C₁-C₁₀ (oxy)alkyl, -C(O)NH₂, -C(O)NHR₁₂, or -aryl;

R₁₁ is -H, -C₁-C₁₀ alkyl, -(C₃-C₇) cycloalkyl, -C₁-C₁₀ (halo)alkyl, -aryl, -C₂-C₁₀ alkenyl, -C₂-C₁₀ alkynyl, -C₁-C₁₀ (aryl)alkyl, -C₂-C₁₀ (aryl)alkenyl, -C₂-C₁₀ (aryl)alkynyl, -C₁-C₁₀ (hydroxy)alkyl, -C₁-C₁₀ alkoxy, -C₁-C₁₀ (amino)alkyl,

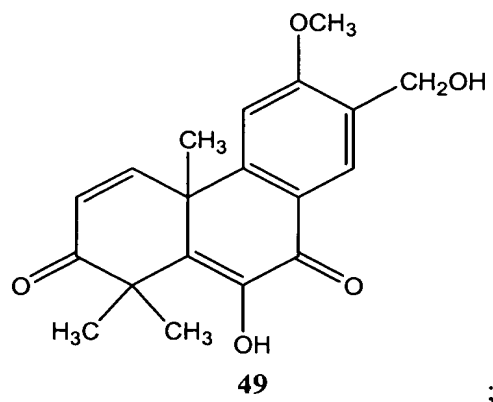
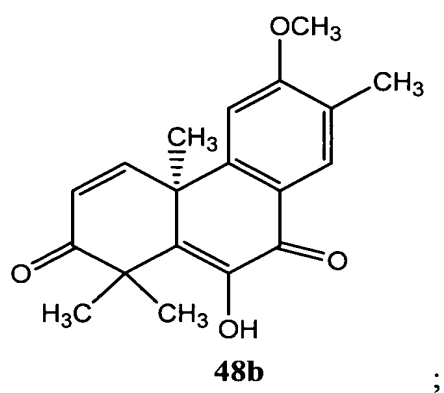
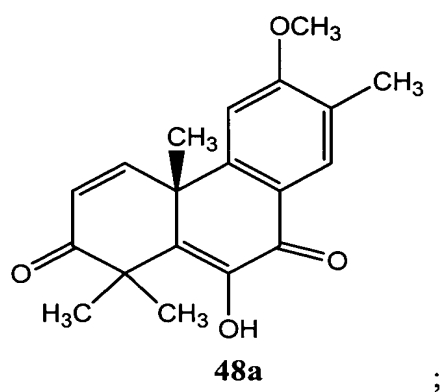
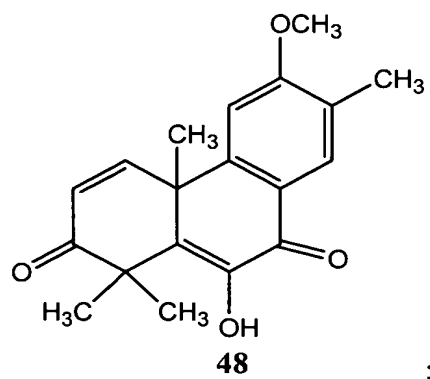
a -(C₃-C₇) cycloalkyl unsubstituted or substituted with one or more -C₁-C₁₀ alkyl, a three- to seven-membered non-aromatic heterocycle unsubstituted or substituted with one or more -C₁-C₁₀ alkyl, or

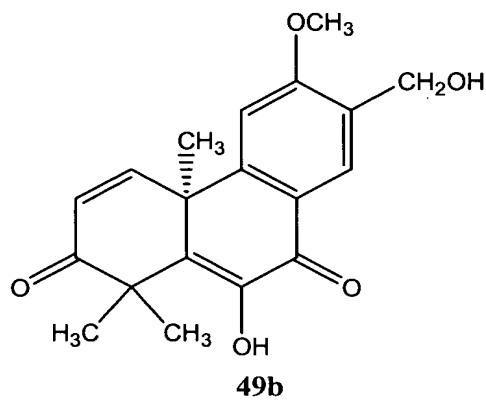
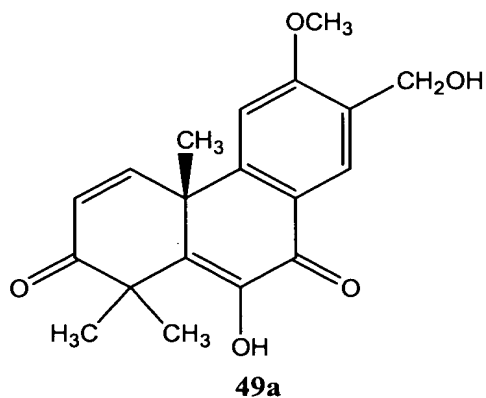
a three- to seven-membered aromatic heterocycle unsubstituted or substituted with one or more -C₁-C₁₀ alkyl, -C₂-C₁₀ alkenyl, or -C₂-C₁₀ alkynyl;

R₁₂ is C₁-C₁₀ alkyl; and

each halogen is independently -F, -Cl, -Br or -I.

In one embodiment, the compound of Formula (I) is not:

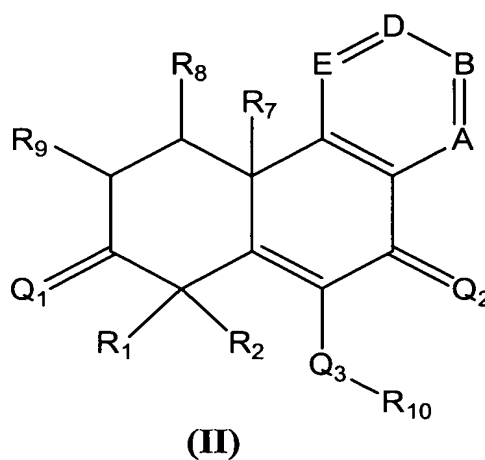




or a pharmaceutically acceptable salt thereof.

In another embodiment, the compound or a pharmaceutically acceptable salt of the compound of Formula (I) is in isolated and purified form.

The present invention still further provides compounds having the Formula (II):



and pharmaceutically acceptable salts thereof, wherein:

Q_1 and Q_2 are independently =O, =S, =NH or =N-NHR, where R is -H, -C₁-C₁₀ alkyl, or -aryl;

Q₃ is -O-, -S-, or -N(H)-;

R₁ and R₂ are independently -H, -halogen, -amino, -C₁-C₁₀ alkyl, -C₁-C₁₀ alkoxy, -C₁-C₁₀ (hydroxy)alkyl, -C₁-C₁₀ (amino)alkyl, -C₁-C₁₀ (halo)alkyl, -C₂-C₁₀ alkenyl, -C₂-C₁₀ alkynyl, (C₃-C₇) cycloalkyl, -aryl, C₁-C₁₀ (aryl)alkyl, or three- to seven-membered non-aromatic heterocycle, or R₁, R₂ and the carbon atom to which they are both attached are taken together to form a (C₃-C₇) cycloalkyl group or a three- to seven-membered non-aromatic heterocycle;

A is N or CR₃; B is N or CR₄; D is N or CR₅; E is N or CR₆, at least one of A, B, D and E being CR₃, CR₄, CR₅ or CR₆, respectively;

each R₃, R₄, R₅ and R₆ is independently -H, -halogen, -CN, -NH₂, -NO₂, -COOH, -C(O)NH₂, -SH, -S(O)NH₂, -S(O)₂NH₂, -C₁-C₁₀ (oxy)alkyl, -C₁-C₁₀ alkyl, -C₁-C₁₀ alkoxy, -C₁-C₁₀ (hydroxy)alkyl, -C₁-C₁₀ (amino)alkyl, -C₁-C₁₀ (halo)alkyl, -C₂-C₁₀ alkenyl, -C₂-C₁₀ alkynyl, -(C₃-C₇) cycloalkyl, -aryl, -C₁-C₁₀ (aryl)alkyl, three- to seven-membered non-aromatic heterocycle, five- to seven-membered aromatic heterocycle, -CH₂OR₁₁, -OCH₂OR₁₁, -OC(O)R₁₁, -C(O)R₁₁, -OC(O)OR₁₁, -OC(O)NR₁₁, -C(O)OR₁₁, -C(O)NR₁₁, -OP(O)(OR₁₁)₂, -SR₁₁, -S(O)₂NHR₁₁, -SOR₁₁, -S(O)₂R₁₁, -NHC(O)R₁₁, -NHSOR₁₁, or NHS(O)₂R₁₁; or

R₃ and R₄ and the carbon atoms to which they are attached are taken together to form a (C₃-C₇) cycloalkenyl group, a five- to seven-membered non-aromatic heterocycle, or a five- to seven-membered aromatic heterocycle; or

R₅ and R₆ and the carbon atoms to which they are attached are taken together to form a (C₃-C₇) cycloalkenyl group, a five- to seven-membered non-aromatic heterocycle, or a five- to seven-membered aromatic heterocycle; or

R₄ and R₅ and the carbon atoms to which they are attached are taken together to form a (C₃-C₇) cycloalkenyl group, a non-oxygen-containing five-membered non-aromatic heterocycle, a non-oxygen-containing five-membered aromatic heterocycle, a six- to seven-membered non-aromatic heterocycle or a six- to seven-membered aromatic heterocycle;

R₇ is -H, -C₁-C₁₀ alkyl, or -C₁-C₁₀ alkoxy;

R₈ and R₉ are each independently -H, -halogen, -CN, -NH₂, -NO₂, -COOH, -C(O)NH₂, -SH, -S(O)NH₂, -S(O)₂NH₂, -C₁-C₁₀ (oxy)alkyl, -C₁-C₁₀ alkyl, -C₁-C₁₀ alkoxy, -C₁-C₁₀ (hydroxy)alkyl, -C₁-C₁₀ (amino)alkyl, -C₁-C₁₀ (halo)alkyl, -C₂-C₁₀ alkenyl, -C₂-C₁₀ alkynyl, -(C₃-C₇) cycloalkyl, -aryl, -C₁-C₁₀ (aryl)alkyl, three- to seven-membered non-aromatic heterocycle, five- to seven-membered aromatic heterocycle, -CH₂OR₁₁, -OCR₁₁,

-OC(O)R₁₁, -C(O)R₁₁, -OC(O)OR₁₁, -OC(O)NR₁₁, -C(O)OR₁₁, -C(O)NR₁₁, -OP(O)(OR₁₁)₂, -SR₁₁, -SOR₁₁, -S(O)₂R₁₁, -S(O)₂NHR₁₁, -NH₂SR₁₁, -NHSOR₁₁, or -NHS(O)₂R₁₁;

R₁₀ is -H, -C₁-C₁₀ alkyl, -C₃-C₇ cycloalkyl, -C(O)C₁-C₁₀ alkyl, -C₁-C₁₀ (oxy)alkyl, -C(O)NH₂, -C(O)NHR₁₂, or -aryl;

R₁₁ is -H, -C₁-C₁₀ alkyl, -(C₃-C₇) cycloalkyl, -C₁-C₁₀ (halo)alkyl, -aryl, -C₂-C₁₀ alkenyl, -C₂-C₁₀ alkynyl, -C₁-C₁₀ (aryl)alkyl, -C₂-C₁₀ (aryl)alkenyl, -C₂-C₁₀ (aryl)alkynyl, -C₁-C₁₀ (hydroxy)alkyl, -C₁-C₁₀ alkoxy, -C₁-C₁₀ (amino)alkyl,

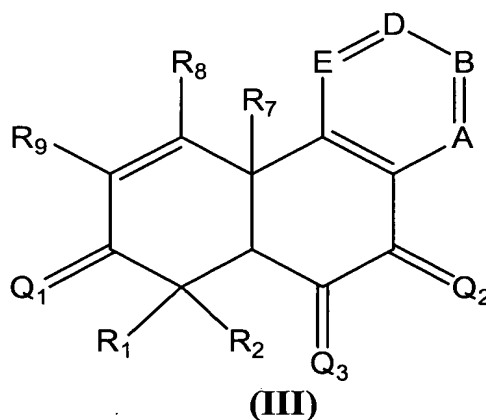
a -(C₃-C₇) cycloalkyl unsubstituted or substituted with one or more -C₁-C₁₀ alkyl, a three- to seven-membered non-aromatic heterocycle unsubstituted or substituted with one or more -C₁-C₁₀ alkyl, or

a three- to seven-membered aromatic heterocycle unsubstituted or substituted with one or more -C₁-C₁₀ alkyl, -C₂-C₁₀ alkenyl, or -C₂-C₁₀ alkynyl;

R₁₂ is C₁-C₁₀ alkyl; and

each halogen is independently -F, -Cl, -Br or -I.

The present invention still further provides compounds having the Formula (III):



and pharmaceutically acceptable salts thereof, wherein:

Q₁, Q₂ and Q₃ are independently =O, =S, =NH or =N-NHR, where R is -H, -C₁-C₁₀ alkyl, or -aryl;

R₁ and R₂ are independently -H, -halogen, -amino, -C₁-C₁₀ alkyl, -C₁-C₁₀ alkoxy, -C₁-C₁₀ (hydroxy)alkyl, -C₁-C₁₀ (amino)alkyl, -C₁-C₁₀ (halo)alkyl, -C₂-C₁₀ alkenyl, -C₂-C₁₀ alkynyl, (C₃-C₇) cycloalkyl, -aryl, C₁-C₁₀ (aryl)alkyl, or three- to seven-membered non-aromatic heterocycle, or R₁, R₂ and the carbon atom to which they are both attached are taken together to form a (C₃-C₇) cycloalkyl group or a three- to seven-membered non-aromatic heterocycle;

A is N or CR₃; B is N or CR₄; D is N or CR₅; E is N or CR₆, at least one of A, B, D and E being CR₃, CR₄, CR₅ or CR₆, respectively;

each R₃, R₄, R₅ and R₆ is independently -H, -halogen, -CN, -NH₂, -NO₂, -COOH, -C(O)NH₂, -SH, -S(O)NH₂, -S(O)₂NH₂, -C₁-C₁₀ (oxy)alkyl, -C₁-C₁₀ alkyl, -C₁-C₁₀ alkoxy, -C₁-C₁₀ (hydroxy)alkyl, -C₁-C₁₀ (amino)alkyl, -C₁-C₁₀ (halo)alkyl, -C₂-C₁₀ alkenyl, -C₂-C₁₀ alkynyl, -(C₃-C₇) cycloalkyl, -aryl, -C₁-C₁₀ (aryl)alkyl, three- to seven-membered non-aromatic heterocycle, five- to seven-membered aromatic heterocycle, -CH₂OR₁₁, -OCH₂OR₁₁, -OC(O)R₁₁, -C(O)R₁₁, -OC(O)OR₁₁, -OC(O)NR₁₁, -C(O)OR₁₁, -C(O)NR₁₁, -OP(O)(OR₁₁)₂, -SR₁₁, -S(O)₂NHR₁₁, -SOR₁₁, -S(O)₂R₁₁, -NHC(O)R₁₁, -NHSOR₁₁, or NHS(O)₂R₁₁; or

R₃ and R₄ and the carbon atoms to which they are attached are taken together to form a (C₃-C₇) cycloalkenyl group, a five- to seven-membered non-aromatic heterocycle, or a five- to seven-membered aromatic heterocycle; or

R₅ and R₆ and the carbon atoms to which they are attached are taken together to form a (C₃-C₇) cycloalkenyl group, a five- to seven-membered non-aromatic heterocycle, or a five- to seven-membered aromatic heterocycle; or

R₄ and R₅ and the carbon atoms to which they are attached are taken together to form a (C₃-C₇) cycloalkenyl group, a non-oxygen-containing five-membered non-aromatic heterocycle, a non-oxygen-containing five-membered aromatic heterocycle, a six- to seven-membered non-aromatic heterocycle or a six- to seven-membered aromatic heterocycle;

R₇ is -H, -C₁-C₁₀ alkyl, or -C₁-C₁₀ alkoxy;

R₈ and R₉ are each independently -H, -halogen, -CN, -NH₂, -NO₂, -COOH, -C(O)NH₂, -SH, -S(O)NH₂, -S(O)₂NH₂, -C₁-C₁₀ (oxy)alkyl, -C₁-C₁₀ alkyl, -C₁-C₁₀ alkoxy, -C₁-C₁₀ (hydroxy)alkyl, -C₁-C₁₀ (amino)alkyl, -C₁-C₁₀ (halo)alkyl, -C₂-C₁₀ alkenyl, -C₂-C₁₀ alkynyl, -(C₃-C₇) cycloalkyl, -aryl, -C₁-C₁₀ (aryl)alkyl, three- to seven-membered non-aromatic heterocycle, five- to seven-membered aromatic heterocycle, -CH₂OR₁₁, -OCR₁₁, -OC(O)R₁₁, -C(O)R₁₁, -OC(O)OR₁₁, -OC(O)NR₁₁, -C(O)OR₁₁, -C(O)NR₁₁, -OP(O)(OR₁₁)₂, -SR₁₁, -SOR₁₁, -S(O)₂R₁₁, -S(O)₂NHR₁₁, -NHSR₁₁, -NHSOR₁₁, or -NHS(O)₂R₁₁;

R₁₁ is -H, -C₁-C₁₀ alkyl, -(C₃-C₇) cycloalkyl, -C₁-C₁₀ (halo)alkyl, -aryl, -C₂-C₁₀ alkenyl, -C₂-C₁₀ alkynyl, -C₁-C₁₀ (aryl)alkyl, -C₂-C₁₀ (aryl)alkenyl, -C₂-C₁₀ (aryl)alkynyl, -C₁-C₁₀ (hydroxy)alkyl, -C₁-C₁₀ alkoxy, -C₁-C₁₀ (amino)alkyl,

a -(C₃-C₇) cycloalkyl unsubstituted or substituted with one or more -C₁-C₁₀ alkyl,

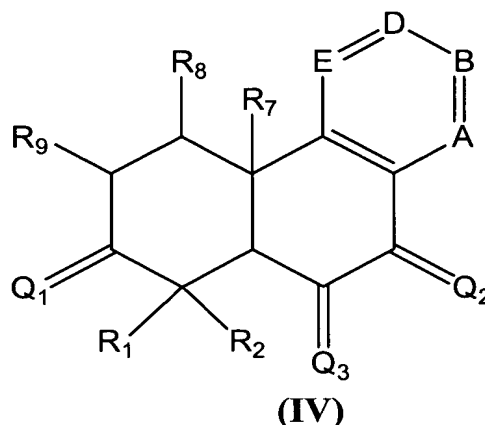
a three- to seven-membered non-aromatic heterocycle unsubstituted or substituted with one or more -C₁-C₁₀ alkyl, or

a three- to seven-membered aromatic heterocycle unsubstituted or substituted with one or more -C₁-C₁₀ alkyl, -C₂-C₁₀ alkenyl, or -C₂-C₁₀ alkynyl;

R₁₂ is C₁-C₁₀ alkyl; and

each halogen is independently -F, -Cl, -Br or -I.

The present invention encompasses compounds having the Formula (IV):



and pharmaceutically acceptable salts thereof, wherein:

Q₁, Q₂ and Q₃ are independently =O, =S, =NH or =N-NHR, where R is -H, -C₁-C₁₀ alkyl, or -aryl;

R₁ and R₂ are independently -H, -halogen, -amino, -C₁-C₁₀ alkyl, -C₁-C₁₀ alkoxy, -C₁-C₁₀ (hydroxy)alkyl, -C₁-C₁₀ (amino)alkyl, -C₁-C₁₀ (halo)alkyl, -C₂-C₁₀ alkenyl, -C₂-C₁₀ alkynyl, (C₃-C₇) cycloalkyl, -aryl, C₁-C₁₀ (aryl)alkyl, or three- to seven-membered non-aromatic heterocycle, or R₁, R₂ and the carbon atom to which they are both attached are taken together to form a (C₃-C₇) cycloalkyl group or a three- to seven-membered non-aromatic heterocycle;

A is N or CR₃; B is N or CR₄; D is N or CR₅; E is N or CR₆, at least one of A, B, D and E being CR₃, CR₄, CR₅ or CR₆, respectively;

each R₃, R₄, R₅ and R₆ is independently -H, -halogen, -CN, -NH₂, -NO₂, -COOH, -C(O)NH₂, -SH, -S(O)NH₂, -S(O)₂NH₂, -C₁-C₁₀ (oxy)alkyl, -C₁-C₁₀ alkyl, -C₁-C₁₀ alkoxy, -C₁-C₁₀ (hydroxy)alkyl, -C₁-C₁₀ (amino)alkyl, -C₁-C₁₀ (halo)alkyl, -C₂-C₁₀ alkenyl, -C₂-C₁₀ alkynyl, -(C₃-C₇) cycloalkyl, -aryl, -C₁-C₁₀ (aryl)alkyl, three- to seven-membered non-aromatic heterocycle, five- to seven-membered aromatic heterocycle, -CH₂OR₁₁, -OCH₂OR₁₁, -OC(O)R₁₁, -C(O)R₁₁, -OC(O)OR₁₁, -OC(O)NR₁₁, -C(O)OR₁₁, -C(O)NR₁₁,

-OP(O)(OR₁₁)₂, -SR₁₁, -S(O)₂NHR₁₁, -SOR₁₁, -S(O)₂R₁₁, -NHC(O)R₁₁, -NHSOR₁₁, or NHS(O)₂R₁₁; or

R₃ and R₄ and the carbon atoms to which they are attached are taken together to form a (C₃-C₇) cycloalkenyl group, a five- to seven-membered non-aromatic heterocycle, or a five- to seven-membered aromatic heterocycle; or

R₅ and R₆ and the carbon atoms to which they are attached are taken together to form a (C₃-C₇) cycloalkenyl group, a five- to seven-membered non-aromatic heterocycle, or a five- to seven-membered aromatic heterocycle; or

R₄ and R₅ and the carbon atoms to which they are attached are taken together to form a (C₃-C₇) cycloalkenyl group, a non-oxygen-containing five-membered non-aromatic heterocycle, a non-oxygen-containing five-membered aromatic heterocycle, a six- to seven-membered non-aromatic heterocycle or a six- to seven-membered aromatic heterocycle;

R₇ is -H, -C₁-C₁₀ alkyl, or -C₁-C₁₀ alkoxy;

R₈ and R₉ are each independently -H, -halogen, -CN, -NH₂, -NO₂, -COOH, -C(O)NH₂, -SH, -S(O)NH₂, -S(O)₂NH₂, -C₁-C₁₀ (oxy)alkyl, -C₁-C₁₀ alkyl, -C₁-C₁₀ alkoxy, -C₁-C₁₀ (hydroxy)alkyl, -C₁-C₁₀ (amino)alkyl, -C₁-C₁₀ (halo)alkyl, -C₂-C₁₀ alkenyl, -C₂-C₁₀ alkynyl, -(C₃-C₇) cycloalkyl, -aryl, -C₁-C₁₀ (aryl)alkyl, three- to seven-membered non-aromatic heterocycle, five- to seven-membered aromatic heterocycle, -CH₂OR₁₁, -OCR₁₁, -OC(O)R₁₁, -C(O)R₁₁, -OC(O)OR₁₁, -OC(O)NR₁₁, -C(O)OR₁₁, -C(O)NR₁₁, -OP(O)(OR₁₁)₂, -SR₁₁, -SOR₁₁, -S(O)₂R₁₁, -S(O)₂NHR₁₁, -NHSR₁₁, -NHSOR₁₁, or -NHS(O)₂R₁₁;

R₁₁ is -H, -C₁-C₁₀ alkyl, -(C₃-C₇) cycloalkyl, -C₁-C₁₀ (halo)alkyl, -aryl, -C₂-C₁₀ alkenyl, -C₂-C₁₀ alkynyl, -C₁-C₁₀ (aryl)alkyl, -C₂-C₁₀ (aryl)alkenyl, -C₂-C₁₀ (aryl)alkynyl, -C₁-C₁₀ (hydroxy)alkyl, -C₁-C₁₀ alkoxy, -C₁-C₁₀ (amino)alkyl,

a -(C₃-C₇) cycloalkyl unsubstituted or substituted with one or more -C₁-C₁₀ alkyl, a three- to seven-membered non-aromatic heterocycle unsubstituted or substituted with one or more -C₁-C₁₀ alkyl, or

a three- to seven-membered aromatic heterocycle unsubstituted or substituted with one or more -C₁-C₁₀ alkyl, -C₂-C₁₀ alkenyl, or -C₂-C₁₀ alkynyl;

R₁₂ is C₁-C₁₀ alkyl; and

each halogen is independently -F, -Cl, -Br or -I.

A compound of Formula (I), Formula (II), Formula (III), or Formula (IV), or a pharmaceutically acceptable salts thereof (a "Diterpenoid Compound") is useful for treating or preventing cancer, a neoplastic disease or a fungal infection in a patient in need of such

treatment or prevention. The Diterpenoid Compounds are also useful for inhibiting the growth of a cancer cell, neoplastic cell or fungus. The Diterpenoid Compounds are also useful for inducing cytotoxicity, *e.g.*, through apoptosis, in a cancer cell or a neoplastic cell.

The present invention provides compositions comprising a pharmaceutically acceptable carrier and an effective amount of a Diterpenoid Compound. The compositions are useful for treating or preventing cancer, neoplastic disease or a fungal infection in a patient in need of such treatment or prevention. These compositions are also useful for inhibiting the growth of a cancer cell, neoplastic cell or fungus. These compositions are further useful for inducing cytotoxicity, *e.g.*, through apoptosis, in a cancer cell or a neoplastic cell.

The invention further provides methods for treating or preventing cancer or a neoplastic disease, comprising administering to a patient in need of such treatment or prevention an effective amount of a Diterpenoid Compound.

The invention further provides methods for inhibiting the growth of a cancer cell or neoplastic cell, comprising contacting the cancer cell or neoplastic cell with an effective amount of a Diterpenoid Compound.

The invention further provides methods for inducing cytotoxicity, *e.g.*, through apoptosis, in a cancer cell or neoplastic cell comprising contacting a cancer cell or neoplastic cell with an effective amount of a Diterpenoid Compound.

The invention further provides methods for inducing apoptosis in a cancer cell or neoplastic cell, comprising contacting a cancer cell or neoplastic cell capable of undergoing apoptosis with an effective amount of a Diterpenoid Compound.

In one embodiment, the Diterpenoid Compound is in isolated and purified form.

The invention further provides methods for treating or preventing a fungal infection, comprising administering to a patient in need of such treatment or prevention an effective amount of a Diterpenoid Compound.

The invention further provides methods for inhibiting the growth of a fungus, comprising contacting the fungus with an effective amount of a Diterpenoid Compound.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A is an isolation scheme for the isolation of Compounds **48** and **49** from dried biomass of *Linum arboreum*; **Fig. 1B** is a summary of the ^1H - ^{13}C correlations observed for Compound **48**.

Fig. 2 is a bar graph depicting Caspase activity in different cell lines following 16 hours of *in vitro* treatment with 1.6 μ M of Compound **48** (10-Hydroxy-6-methoxy-1,1,4a,7-tetramethyl-1H,4aH-phenanthrene-2,9-dione).

Fig. 3 is a bar graph depicting cell survival of different cell types following 72 hours of *in vitro* treatment with Compound **48** (10-Hydroxy-6-methoxy-1,1,4a,7-tetramethyl-1H,4aH-phenanthrene-2,9-dione).

Fig. 4 is a graph depicting the weight of mice following treatment with Cisplatin or Compound **50**. The time-points of administration of Cisplatin or Compound **50** are indicated by arrows on top of the graph.

Fig. 5 is a graph depicting the tumor size in mice following treatment with Cisplatin or Compound **50**. The time-points of administration of Cisplatin or Compound **50** are indicated by arrows on top of the graph.

Fig. 6 is a graph depicting the net growth of *Saccharomyces cerevisiae* following treatment with Compound **50**.

Fig. 7 is a graph depicting the net growth of *Candida albicans* following treatment with Compound **50**.

5. DETAILED DESCRIPTION OF THE INVENTION

5.1 DEFINITIONS AND ABBREVIATIONS

As used herein, the term “C₁-C₁₀ alkyl” means a saturated straight chain or branched non-cyclic hydrocarbon having from 1 to 10 carbon atoms. Representative saturated straight chain alkyls include -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, -n-hexyl, -n-heptyl, -n-octyl, -n-nonyl and -n-decyl; while saturated branched alkyls include -isopropyl, -*sec*-butyl, -isobutyl, -*tert*-butyl, -isopentyl, -2-methylbutyl, 3-methylbutyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 2-methylhexyl, 3-methylhexyl, 4-methylhexyl, 5-methylhexyl, 2,3-dimethylbutyl, 2,3-dimethylpentyl, 2,4-dimethylpentyl, 2,3-dimethylhexyl, 2,4-dimethylhexyl, 2,5-dimethylhexyl, 2,2-dimethylpentyl, 2,2-dimethylhexyl, 3,3-dimethylpentyl, 3,3-dimethylhexyl, 4,4-dimethylhexyl, 2-ethylpentyl, 3-ethylpentyl, 2-ethylhexyl, 3-ethylhexyl, 4-ethylhexyl, 2-methyl-2-ethylpentyl, 2-methyl-3-ethylpentyl, 2-methyl-4-ethylpentyl, 2-methyl-2-ethylhexyl, 2-methyl-3-ethylhexyl, 2-methyl-4-ethylhexyl, 2,2-diethylpentyl, 3,3-diethylhexyl, 2,2-diethylhexyl, 3,3-diethylhexyl and the like.

As used herein, the term “C₁-C₁₀ alkoxy” means -O-(C₁-C₁₀ alkyl), wherein C₁-C₁₀ alkyl is defined above.

As used herein, the term “C₁-C₁₀ (hydroxy)alkyl” means C₁-C₁₀ alkyl, wherein C₁-C₁₀ alkyl is defined above, substituted with one or more -OH groups. Examples of C₁-C₁₀ (hydroxy)alkyl include, but are not limited to, hydroxymethyl, 1-hydroxyethyl, 2-hydroxyethyl, 1-hydroxypropyl, 2-hydroxypropyl, 3-hydroxypropyl, 4-hydroxybutyl, 5-hydroxypentyl and the like.

As used herein, the term “C₁-C₁₀ (amino)alkyl” means C₁-C₁₀ alkyl, wherein C₁-C₁₀ alkyl is defined above, substituted with one or more -NH₂ groups. Examples of C₁-C₁₀ (amino)alkyl include, but are not limited to, -CH₂-NH₂, -(CH₂)₂-NH₂, -(CH₂)₃-NH₂, -(CH₂)₄-NH₂, -(CH₂)₅-NH₂ and the like.

As used herein, the term “C₁-C₁₀ (halo)alkyl” means C₁-C₁₀ alkyl, wherein C₁-C₁₀ alkyl is defined above, substituted with one or more -F, -Cl, Br or -I groups. Examples of C₁-C₁₀ (halo)alkyl include, but are not limited to, trichloromethyl, trifluoromethyl, dichloromethyl, difluoromethyl, 1-fluoroethyl, 2-chloroethyl, 1-bromopropyl, 2-iodopropyl, 3-chloropropyl, 4-fluorobutyl, 5-chloropentyl and the like.

As used herein, the term “C₂-C₁₀ alkenyl” means a straight chain or branched non-cyclic hydrocarbon having from 2 to 10 carbon atoms and including at least one carbon-carbon double bond. Representative straight chain and branched C₂-C₁₀ alkenyls include -vinyl, -allyl, -1-butenyl, -2-butenyl, -isobutylenyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, -2,3-dimethyl-2-butenyl, -1-hexenyl, -2-hexenyl, -3-hexenyl, -1-heptenyl, -2-heptenyl, -3-heptenyl, -1-octenyl, -2-octenyl, -3-octenyl, -1-nonenyl, -2-nonenyl, -3-nonenyl, -1-decenyl, -2-decenyl, -3-decenyl and the like. In one embodiment, C₂-C₆ alkenyl is a subclass of C₂-C₁₀ alkenyl. The double bond of a C₂-C₁₀ alkenyl can be unconjugated or conjugated to another unsaturated group. A -C₂-C₁₀ alkenyl can be unsubstituted or substituted with, *e.g.*, -amino, -C₁-C₁₀ (oxy)alkyl, -halogen, -COOH, -C(O)C₁-C₉ alkyl, -SH, =S, -OH, and -C₁-C₁₀ alkoxy.

As used herein, unless otherwise specified the term “C₂-C₁₀ alkynyl” means a straight chain or branched non-cyclic hydrocarbon having from 2-10 carbon atoms and including at least one carbon-carbon triple bond. Representative straight chain and branched C₂-C₁₀ alkynyls include -acetylenyl, -propynyl, -1-butyne, -2-butyne, -1-pentyne, -2-pentyne, -3-methyl-1-butyne, -4-pentyne, -1-hexyne, -2-hexyne, -5-hexyne, -1-heptyne, -2-heptyne, -6-heptyne, -1-octyne, -2-octyne, -7-octyne, -1-nonyne, -2-nonyne, -8-nonyne, -1-decynyl, -2-decynyl, -9-decynyl, and the like. In one embodiment, C₂-C₆ alkynyl is a subclass of C₂-C₁₀ alkynyl. The triple bond of a C₂-C₁₀ alkynyl can be unconjugated or

conjugated to another unsaturated group. A C₂-C₁₀ alkynyl can be unsubstituted or substituted with, *e.g.*, -amino, -COOH, -halogen, C₁-C₁₀ (oxy)alkyl, -C(O)C₁-C₉ alkyl, -SH, =S, -OH, C₁-C₁₀ alkoxy, and C₁-C₁₀ alkyl.

As used herein, the term “(C₃-C₇) cycloalkyl” means a monocyclic or bicyclic saturated ring consisting of carbon and hydrogen atoms and having 3-7 carbon atoms. A (C₃-C₇) cycloalkyl can be unsubstituted or substituted with, *e.g.*, -amino, -COOH, -halogen, C₁-C₁₀ (oxy)alkyl, -C(O)C₁-C₉ alkyl, -SH, =S, -OH, C₁-C₁₀ alkoxy, and C₁-C₁₀ alkyl. Examples of (C₃-C₇) cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl, and saturated cyclic and bicyclic terpenes.

As used herein, the term “(C₃-C₇) cycloalkenyl” means a monocyclic or bicyclic unsaturated ring consisting of carbon and hydrogen atoms and having 3-7 carbon atoms. A (C₃-C₇) cycloalkenyl can be unsubstituted or substituted with, *e.g.*, -amino, -COOH, -halogen, C₁-C₁₀ (oxy)alkyl, -C(O)C₁-C₉ alkyl, -SH, =S, -OH, C₁-C₁₀ alkoxy, and C₁-C₁₀ alkyl. Examples of (C₃-C₇) cycloalkenyl include, but are not limited to, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, and cycloheptenyl, and unsaturated cyclic and bicyclic terpenes.

As used herein, the term “aryl” means a carbocyclic aromatic group. All of the ring atoms of an aryl group are carbon atoms. Aryl groups include compounds having one or more ring structures such as mono-, bi-, or tricyclic compounds as well as benzo-fused carbocyclic moieties such as 5,6,7,8-tetrahydronaphthyl and the like. In one embodiment, the aryl group is a monocyclic ring or bicyclic ring. Representative aryl groups include phenyl, tolyl, anthryl, fluorenyl, indenyl, azulenyl, phenanthryl and naphthyl. A carbocyclic aryl group can be unsubstituted or substituted with, *e.g.*, -amino, -COOH, -halogen, C₁-C₁₀ (oxy)alkyl, -C(O)C₁-C₉ alkyl, -SH, =S, -OH, C₁-C₁₀ alkoxy, and C₁-C₁₀ alkyl.

As used herein, the term “C₁-C₁₀ (aryl)alkyl” means C₁-C₁₀ alkyl, wherein C₁-C₁₀ alkyl is defined above, substituted with one or more aryl groups, wherein aryl is defined above. Examples of C₁-C₁₀ (aryl)alkyl include, but not limited to -(CH₂)phenyl, -(CH₂)₂phenyl, -(CH₂)₃phenyl, -CH(phenyl)₂, -CH(phenyl)₃, -(CH₂)tolyl, -(CH₂)anthracenyl, -(CH₂)fluorenyl, -(CH₂)indenyl, -(CH₂)azulenyl, -(CH₂)naphthyl, and the like.

As used herein, the term “C₂-C₁₀ (aryl)alkenyl” means C₂-C₁₀ alkenyl, wherein C₂-C₁₀ alkenyl is defined above, substituted with one or more aryl groups, wherein aryl is defined above.

As used herein, the term “C₂-C₁₀ (aryl)alkynyl” means C₂-C₁₀ alkynyl, wherein C₂-C₁₀ alkynyl is defined above, substituted with one or more aryl groups, wherein aryl is defined above.

As used herein, the term “three- to seven-membered aromatic heterocycle” means a heterocyclic ring that contains 3 to 7 ring atoms and that is aromatic. A three-membered heterocycle can contain up to 3 heteroatoms, and a 4- to 7-membered heterocycle can contain up to 4 heteroatoms, wherein the remaining atoms are carbon atoms. Each heteroatom is independently selected from nitrogen, which can be quaternized; oxygen; phosphorus and sulfur, including sulfoxide and sulfone. The heterocycle can be attached via any heteroatom or carbon atom. Representative three- to seven-membered aromatic heterocycles include, but are not limited to, pyridyl, furyl, thiophenyl, pyrrolyl, oxazolyl, imidazolyl, thiazolyl, isoxazolyl, pyrazolyl, isothiazolyl, pyridazinyl, pyrimidinyl, pyrazinyl, and triazinyl.

As used herein, the term “three- to seven-membered non-aromatic heterocycle” means a heterocyclic ring that contains 3 to 7 ring atoms and that is non-aromatic. A three-membered heterocycle can contain up to 3 heteroatoms, and a 4- to 7-membered heterocycle can contain up to 4 heteroatoms, wherein the remaining atoms are carbon atoms. Each heteroatom is independently selected from nitrogen, which can be quaternized; oxygen; phosphorus; and sulfur, including sulfoxide and sulfone. The heterocycle can be attached via any heteroatom or carbon atom. Representative three- to seven-membered non-aromatic heterocycles include, but are not limited to, morpholinyl, pyrrolidinonyl, pyrrolidinyl, piperidinyl, piperazinyl, hydantoinyl, valerolactamyl, oxiranyl, oxetanyl, pyranyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydropyridinyl, tetrahydropyrimidinyl, tetrahydrothiophenyl, and tetrahydrothiopyranyl.

As used herein, the term “five- to seven-membered aromatic heterocycle” means a heterocyclic ring that contains 5 to 7 ring atoms and that is aromatic. A five- to seven-membered heterocycle can contain up to 4 heteroatoms, wherein the remaining atoms are carbon atoms. Each heteroatom is independently selected from nitrogen, which can be quaternized; oxygen; phosphorus; and sulfur, including sulfoxide and sulfone. The heterocycle can be attached via any heteroatom or carbon atom. Representative five- to seven-membered aromatic heterocycles include, but are not limited to, pyridyl, furyl, thiophenyl, pyrrolyl, furazanyl, oxazolyl, imidazolyl, thiazolyl, thiadiazolyl, isoxazolyl, pyrazolyl, isothiazolyl, pyridazinyl, pyrimidinyl, pyrazinyl, and triazinyl.

As used herein, the term “five- to seven-membered non-aromatic heterocycle” means a heterocyclic ring that contains 5 to 7 ring atoms and that is non-aromatic. A five- to seven-membered heterocycle can contain up to 4 heteroatoms, wherein the remaining atoms are carbon atoms. Each heteroatom is independently selected from nitrogen, which can be quaternized; oxygen; phosphorus; and sulfur, including sulfoxide and sulfone. The heterocycle can be attached via any heteroatom or carbon atom. Representative five- to seven-membered non-aromatic heterocycles include, but are not limited to, morpholinyl, pyranyl, pyrrolidinonyl, pyrrolidinyl, piperidinyl, piperazinyl, hydantoinyl, valerolactamyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydropyridinyl, tetrahydropyrimidinyl, tetrahydrothiophenyl, and tetrahydrothiopyranyl.

As used herein, the term “non-oxygen-containing five-membered non-aromatic heterocycle” means a heterocyclic ring that contains 5 ring atoms and that is non-aromatic. A five-membered heterocycle can contain up to 4 heteroatoms, wherein the remaining atoms are carbon atoms. Each heteroatom is independently selected from nitrogen, which can be quaternized; phosphorus; and sulfur, including sulfoxide and sulfone. The heterocycle can be attached via any heteroatom or carbon atom.

As used herein, the term “non-oxygen-containing five-membered aromatic heterocycle” means a heterocyclic ring that contains 5 ring atoms and that is aromatic. A five-membered heterocycle can contain up to 4 heteroatoms, wherein the remaining atoms are carbon atoms. Each heteroatom is independently selected from nitrogen, which can be quaternized; phosphorus; and sulfur, including sulfoxide and sulfone. The heterocycle can be attached via any heteroatom or carbon atom.

Examples of “halogen” are fluorine, chlorine, bromine, and iodine.

As used herein, the term “C₁-C₁₀ (oxy)alkyl” means C₁-C₁₀ alkyl, wherein C₁-C₁₀ alkyl is defined above, and wherein one of its carbon atoms is a C=O group. Examples of C₁-C₁₀ (oxy)alkyl include, but are not limited to, -C(O)CH₃, -CH₂CHO, -C(O)(CH₂)₂CH₃, -CH₂C(O)CH₃, -(CH₂)₂CHO, -(CH₂)₃CHO, -(CH₂)₄CHO and the like.

As used herein, an “effective amount” when used in connection with a Diterpenoid Compound refers to that amount of the Diterpenoid Compound useful for treating or preventing cancer, a neoplastic disease or a fungal infection; for inhibiting the growth of a cancer cell, neoplastic cell or fungus; or for inducing cytotoxicity, *e.g.*, through apoptosis, in a cancer cell or a neoplastic cell, alone or in combination with another active agent. As used herein, an “effective amount” when used in connection with another active agent refers to

that amount of the other active agent that is useful for treating or preventing a particular disease or condition, alone or in combination with a Diterpenoid Compound.

As used herein, the term “treating cancer or a neoplastic disease” includes reducing the size of a tumor, ameliorating one or more symptoms associated with a cancer or a neoplastic disease, or inducing cytotoxicity, *e.g.*, through apoptosis, selectively in cells of a cancer or neoplastic disease relative to a non-cancerous or non-neoplastic cell. The term “treating a cancer or a neoplastic disease” further includes arresting or retarding the progression of a cancer or a neoplastic disease.

As used herein, the term “pharmaceutically acceptable salt” refers to a salt prepared from an acid or a base including inorganic acids and bases and organic acids and bases. Suitable pharmaceutically acceptable salts of a Diterpenoid Compound having a -COOH group include, but are not limited to, metallic salts of aluminum, calcium, lithium, magnesium, potassium, sodium and zinc, or organic salts of lysine, N,N'-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. Acids useful for forming suitable salts with a Diterpenoid Compound having a nitrogen or sulfur atom include, but are not limited to, inorganic and organic acids such as acetic, alginic, anthranilic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethenesulfonic, formic, fumaric, furoic, galacturonic, gluconic, glucuronic, glutamic, glycolic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phenylacetic, phosphoric, propionic, salicylic, stearic, succinic, sulfanilic, sulfuric, tartaric acid, and p-toluenesulfonic acid. Other examples of salts are well known in the art, *see, e.g., Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing, Easton PA (1990).

As used herein, the term “isolated and purified” means isolated from another component or from other components of a naturally occurring source (such as a plant or animal cell, including a hepatocyte; cell culture; tissue; *in vivo* fluid including intracellular and extracellular fluid, including blood and plasma; and *ex vivo* fluid including sputum, urine, sweat, semen, menstrual fluid, and milk) or from a synthetic organic chemical reaction mixture, and processed through one or more purifying steps that separate the compound of the invention from other molecules associated with it. When isolated and purified, the compound of the invention is at least about 95% pure. In one embodiment, the compound of the invention is at least about 98% pure. In another embodiment, the compound of the invention is at least about 99% pure.

When a first group is substituted with "one or more" second group(s), a hydrogen of the first group is replaced with the second group. In one embodiment, a first group is substituted with one, two or three second groups. In another embodiment, a first group is substituted with one or two second groups. In even another embodiment, a first group is substituted with one second group.

As used herein, the term "patient" refers preferably to an animal, including, but not limited, to a vertebrate such a chimpanzee, baboon, cow, horse, sheep, pig, chicken, turkey, quail, cat, dog, mouse, rat, rabbit, and guinea pig, and in one embodiment a mammal, and in a more specific embodiment a human.

A Diterpenoid Compound can have one or more chiral centers and, accordingly, can exist in the form of a diastereomer, a (+)- or (-)-enantiomer, a racemate, or a mixture thereof.

Abbreviations

IBX	iodoxybenzoate
TBAF	tetra- n -butylammonium fluoride
BRDU	bromodeoxyuridine
i.v.	intravenous
Rpm	revolutions per minute
ATCC	American Type Culture Collection

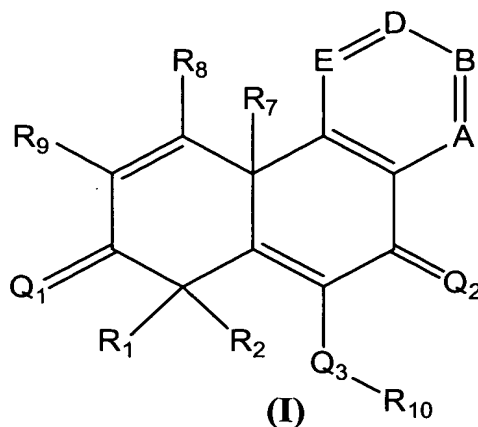
The Diterpenoid Compounds are defined herein by their chemical structures and/or chemical names. Where a Diterpenoid Compound is referred to by both a chemical structure and a chemical name, and the chemical structure and chemical name conflict, the chemical structure is determinative of the compound's identity.

The Diterpenoid Compounds can exist in the form of a pharmaceutically acceptable salt, free base, solvate, hydrate, stereoisomer, clathrate, polymorph or prodrug thereof.

The invention can be understood more fully by reference to the following description, figures and illustrative examples, which are intended to exemplify non-limiting embodiments of the invention.

5.2 FORMULA I

As stated above, the present invention encompasses compounds having the Formula (I):



and pharmaceutically acceptable salts thereof, wherein Q_1 - Q_3 , R_1 - R_{10} , A-E and halogen are defined above for Formula (I).

The Diterpenoid Compounds of Formula (I) are useful for treating or preventing cancer or neoplastic disease in a patient in need of such treatment or prevention. The Diterpenoid Compounds of Formula (I) are also useful for inhibiting the growth of a cancer cell or neoplastic cell. The Diterpenoid Compounds of Formula (I) are also useful for inducing cytotoxicity, *e.g.*, through apoptosis, in a cancer cell or neoplastic cell. The Diterpenoid Compounds of Formula (I) are further useful for treating or preventing a fungal infection. The Diterpenoid Compounds of Formula (I) also useful for inhibiting the growth of a fungus.

In one embodiment, the Diterpenoid Compounds of Formula (I) are those wherein Q_1 and Q_2 are =O and Q_3 is -O-.

In another embodiment, the Diterpenoid Compounds of Formula (I) are those wherein A, B, D and E are CR_3 , CR_4 , CR_5 and CR_6 , respectively.

In another embodiment, the Diterpenoid Compounds of Formula (I) are those wherein A is CR_3 , E is CR_6 , and R_3 and R_6 are hydrogen.

In another embodiment, the Diterpenoid Compounds of Formula (I) are those wherein D is CR_5 and R_5 is C_1 - C_6 alkoxy, in another embodiment, $-OCH_3$.

In another embodiment, the Diterpenoid Compounds of Formula (I) are those wherein B is CR_4 and R_4 is hydrogen.

In another embodiment, the Diterpenoid Compounds of Formula (I) are those wherein B is CR_4 , R_4 is hydrogen, D is CR_5 and R_5 is C_1 - C_6 alkoxy.

In another embodiment, the Diterpenoid Compounds of Formula (I) are those wherein B is CR_4 , R_4 is hydrogen, D is CR_5 and R_5 is $-OCH_3$.

In another embodiment, the Diterpenoid Compounds of Formula (I) are those wherein B is CR₄ and R₄ is C₁-C₁₀ alkyl, in another embodiment, -CH₃.

In another embodiment, the Diterpenoid Compounds of Formula (I) are those wherein B is CR₄ and R₄ is C₁-C₁₀ (hydroxy)alkyl, in another embodiment, hydroxymethyl.

In another embodiment, R₈ and R₉ are hydrogen.

In another embodiment, R₁ and R₂ are independently C₁-C₁₀ alkyl, in another embodiment, -CH₃.

In another embodiment, R₁₀ is hydrogen.

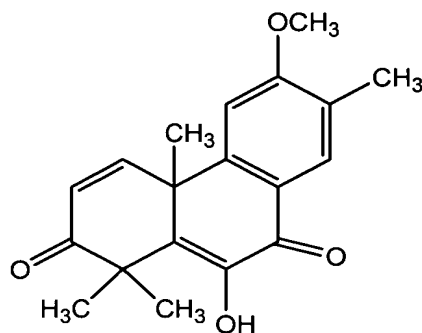
In another embodiment, -Q₃-R₁₀ is -OH.

In another embodiment, R₇ is C₁-C₁₀ alkyl, in another embodiment -CH₃.

In one embodiment, Q₁ and Q₂ and Q₃ are oxygen. In another embodiment, R₁ and R₂ are C₁-C₁₀ alkyl. In another embodiment, R₈ and R₉ are H. In another embodiment, R₇ is C₁-C₁₀ alkyl. In another embodiment, R₃ and R₆ are H. In another embodiment, R₄ and R₅ are independently C₁-C₁₀ alkyl, C₁-C₁₀ alkoxy, or C₁-C₁₀ (hydroxy)alkyl.

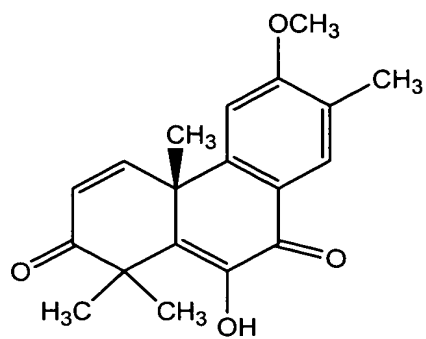
In one embodiment, the Diterpenoid Compounds of Formula (I) are in isolated and purified form.

Illustrative Diterpenoid Compound of Formula (I) are:



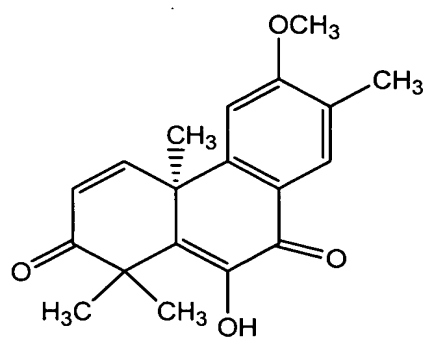
48

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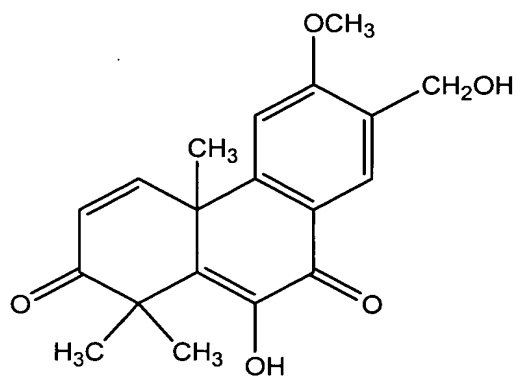
48a

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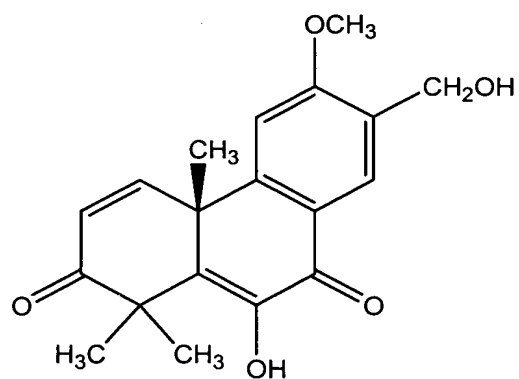
48b

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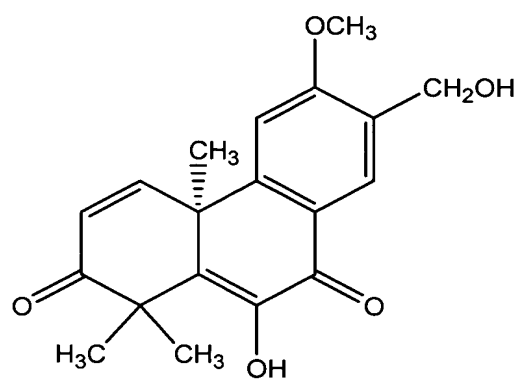
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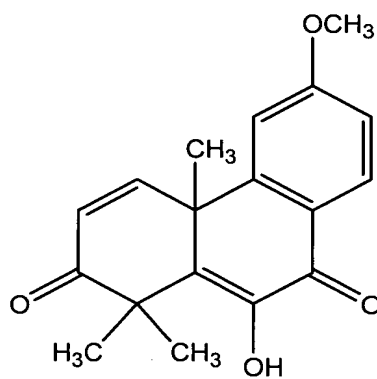
49a

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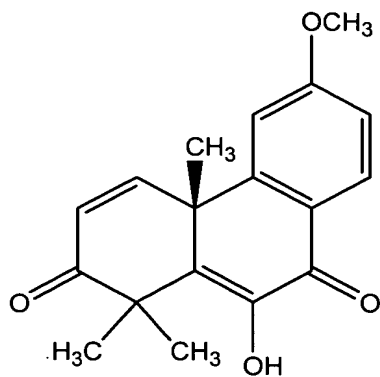
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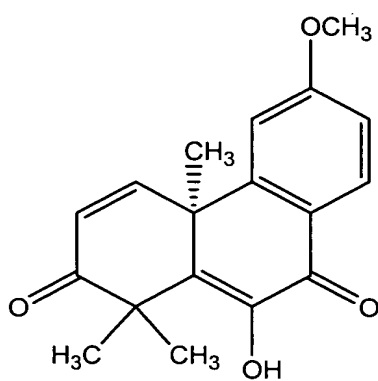
50

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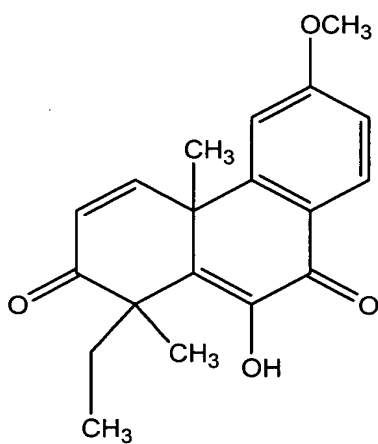
50a

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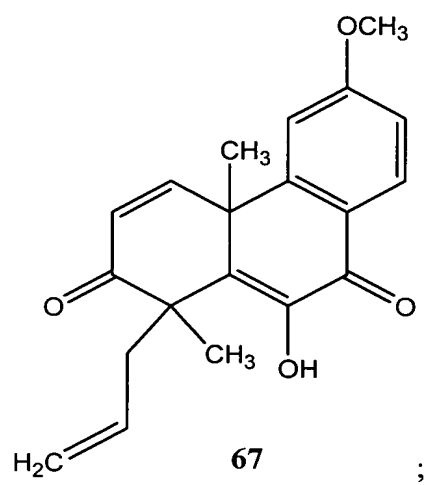
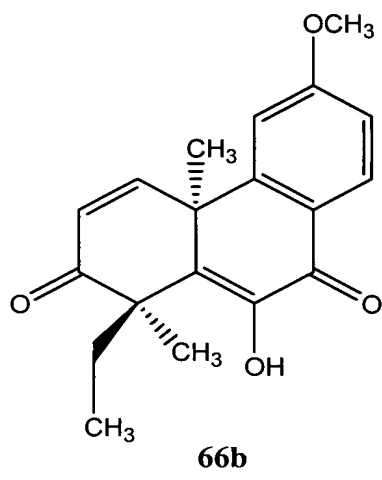
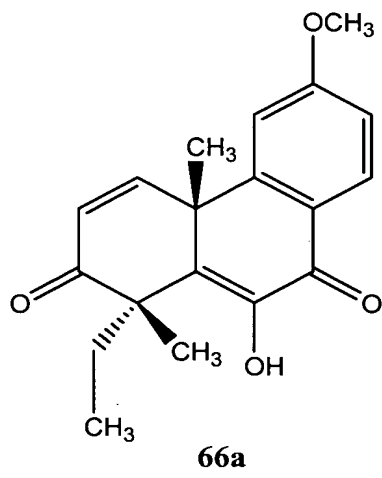
50b

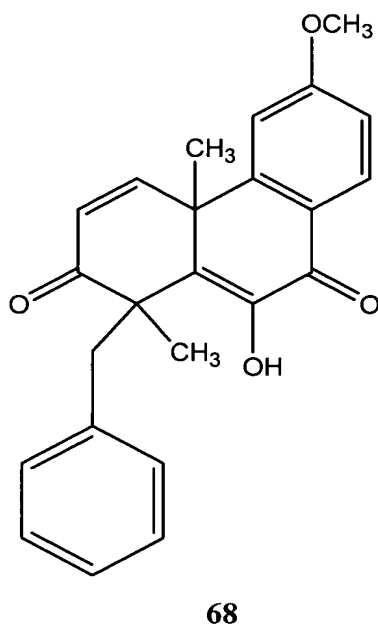
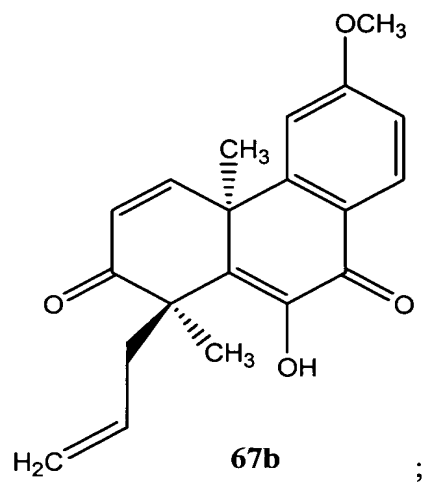
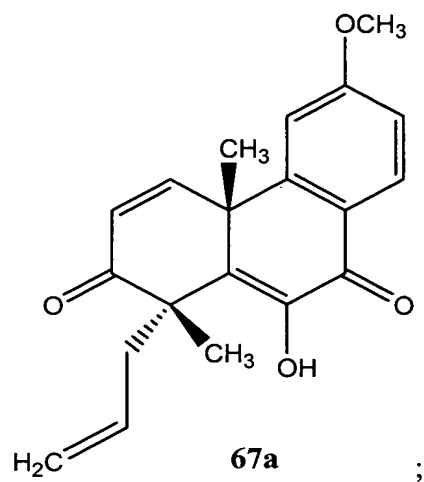
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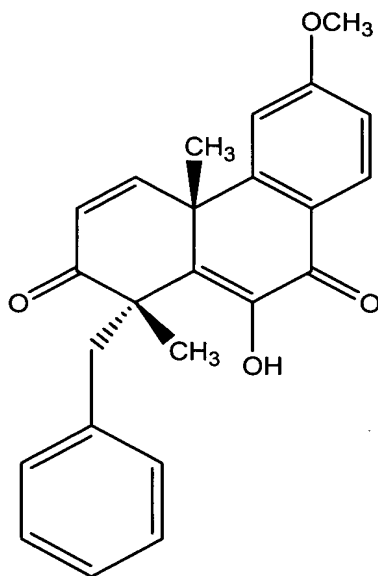


66

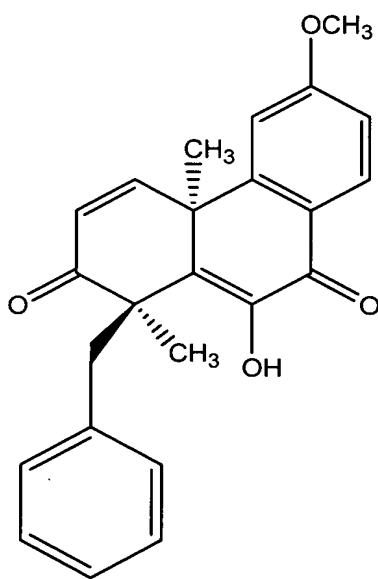
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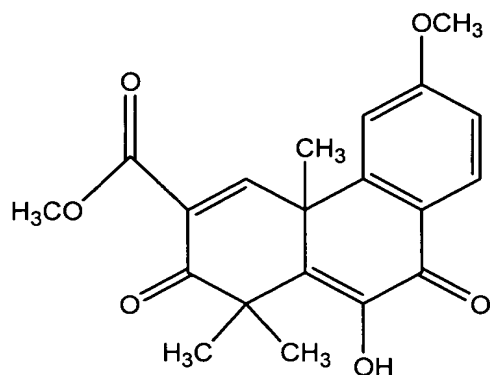




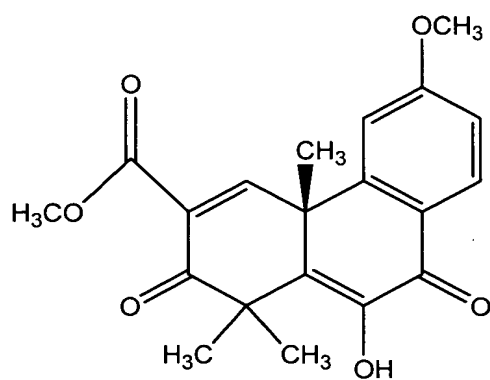
68a ;



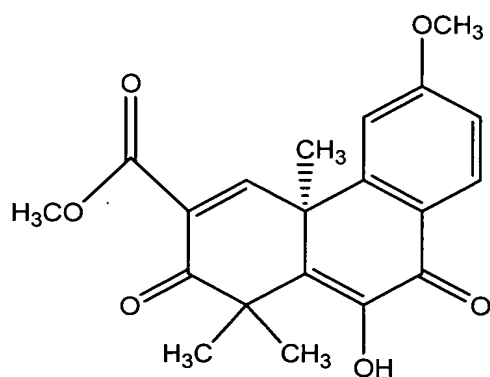
68b ;



69 ;



69a ;



69b ;

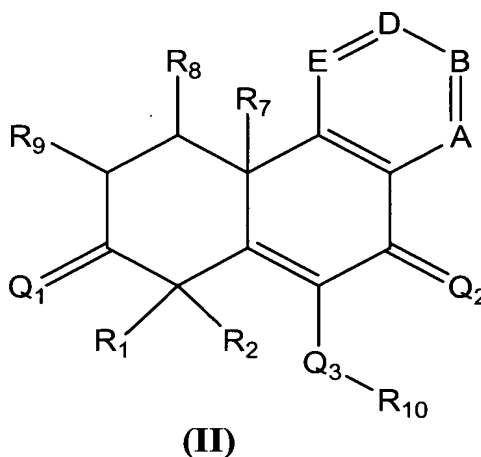
and pharmaceutically acceptable salts thereof.

It is to be understood that Compound **50** is the racemate of its corresponding enantiomers Compound **50a** and **50b**; Compound **66** is the racemate of its corresponding enantiomers Compound **66a** and **66b**; Compound **67** is the racemate of its corresponding

enantiomers Compound **67a** and **67b**; Compound **68** is the racemate of its corresponding enantiomers Compound **68a** and **68b**; and that Compound **69** is the racemate of its corresponding enantiomers Compound **69a** and **69b**.

5.3 FORMULA II

As stated above, the present invention encompasses compounds having the Formula (II):



and pharmaceutically acceptable salts thereof, wherein $Q_1 - Q_3$, $R_1 - R_{10}$, A-E and halogen are defined above for Formula (II).

The Diterpenoid Compounds of Formula (II) are useful for treating or preventing cancer or neoplastic disease in a patient in need of such treatment or prevention. The Diterpenoid Compounds of Formula (II) are also useful for inhibiting the growth of a cancer cell or neoplastic cell. The Diterpenoid Compounds of Formula (II) are also useful for inducing cytotoxicity, *e.g.*, through apoptosis, in a cancer cell or neoplastic cell. The Diterpenoid Compounds of Formula (II) are further useful for treating or preventing a fungal infection. The Diterpenoid Compounds of Formula (II) also useful for inhibiting the growth of a fungus.

In one embodiment, the Diterpenoid Compounds of Formula (II) are those wherein Q_1 and Q_2 are =O and Q_3 is -O-.

In another embodiment, the Diterpenoid Compounds of Formula (II) are those wherein A, B, D and E are CR_3 , CR_4 , CR_5 and CR_6 , respectively.

In another embodiment, the Diterpenoid Compounds of Formula (II) are those wherein A is CR_3 , E is CR_6 , and R_3 and R_6 are hydrogen.

In another embodiment, the Diterpenoid Compounds of Formula (II) are those wherein D is CR₅ and R₅ is C₁-C₆ alkoxy, in another embodiment, -OCH₃.

In another embodiment, the Diterpenoid Compounds of Formula (II) are those wherein B is CR₄ and R₄ is hydrogen.

In another embodiment, the Diterpenoid Compounds of Formula (II) are those wherein B is CR₄, R₄ is hydrogen, D is CR₅ and R₅ is C₁-C₆ alkoxy.

In another embodiment, the Diterpenoid Compounds of Formula (II) are those wherein B is CR₄, R₄ is hydrogen, D is CR₅ and R₅ is -OCH₃.

In another embodiment, the Diterpenoid Compounds of Formula (II) are those wherein B is CR₄ and R₄ is C₁-C₁₀ alkyl, in another embodiment, -CH₃.

In another embodiment, the Diterpenoid Compounds of Formula (II) are those wherein B is CR₄ and R₄ is C₁-C₁₀ (hydroxy)alkyl, in another embodiment, hydroxymethyl.

In another embodiment, R₈ and R₉ are hydrogen.

In another embodiment, R₁ and R₂ are independently C₁-C₁₀ alkyl, in another embodiment, -CH₃.

In another embodiment, R₁₀ is hydrogen.

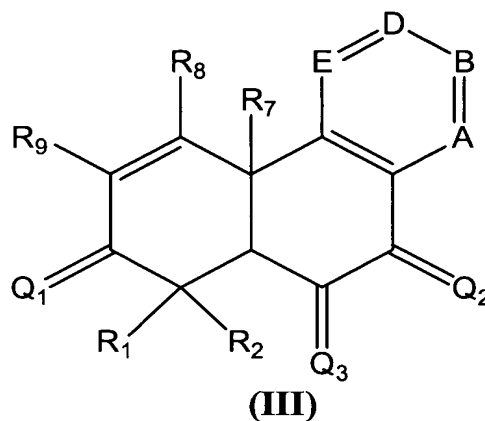
In another embodiment, -Q₃-R₁₀ is -OH.

In another embodiment, R₇ is C₁-C₁₀ alkyl, in another embodiment -CH₃.

In another embodiment, the Diterpenoid Compounds of Formula (II) are in isolated and purified form.

5.4 FORMULA III

As stated above, the present invention encompasses novel compounds having the Formula (III):



and pharmaceutically acceptable salts thereof, wherein $Q_1 - Q_3$, $R_1 - R_9$, A-E and halogen are defined above for Formula (III).

The Diterpenoid Compounds of Formula (III) are useful for treating or preventing cancer or neoplastic disease in a patient in need of such treatment or prevention. The Diterpenoid Compounds of Formula (III) are also useful for inhibiting the growth of a cancer cell or neoplastic cell. The Diterpenoid Compounds of Formula (III) are also useful for inducing cytotoxicity, *e.g.*, through apoptosis, in a cancer cell or neoplastic cell. The Diterpenoid Compounds of Formula (III) are further useful for treating or preventing a fungal infection. The Diterpenoid Compounds of Formula (III) also useful for inhibiting the growth of a fungus.

In one embodiment, the Diterpenoid Compounds of Formula (III) are those wherein Q_1 , Q_2 and Q_3 are =O.

In another embodiment, the Diterpenoid Compounds of Formula (III) are those wherein A, B, D and E are CR_3 , CR_4 , CR_5 and CR_6 , respectively.

In another embodiment, the Diterpenoid Compounds of Formula (III) are those wherein A is CR_3 , E is CR_6 , and R_3 and R_6 are hydrogen.

In another embodiment, the Diterpenoid Compounds of Formula (III) are those wherein D is CR_5 and R_5 is C_1 - C_6 alkoxy, in another embodiment, $-OCH_3$.

In another embodiment, the Diterpenoid Compounds of Formula (III) are those wherein B is CR_4 and R_4 is hydrogen.

In another embodiment, the Diterpenoid Compounds of Formula (III) are those wherein B is CR_4 , R_4 is hydrogen, D is CR_5 and R_5 is C_1 - C_6 alkoxy.

In another embodiment, the Diterpenoid Compounds of Formula (III) are those wherein B is CR_4 , R_4 is hydrogen, D is CR_5 and R_5 is $-OCH_3$.

In another embodiment, the Diterpenoid Compounds of Formula (III) are those wherein B is CR₄ and R₄ is C₁-C₁₀ alkyl, in another embodiment, -CH₃.

In another embodiment, the Diterpenoid Compounds of Formula (III) are those wherein B is CR₄ and R₄ is C₁-C₁₀ (hydroxy)alkyl, in another embodiment, hydroxymethyl.

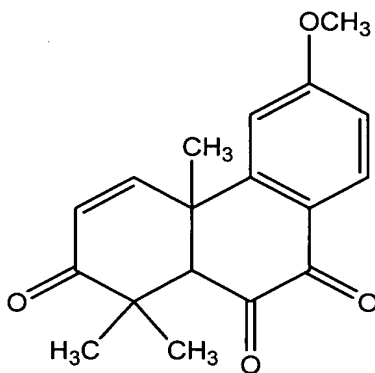
In another embodiment, R₈ and R₉ are hydrogen.

In another embodiment, R₁ and R₂ are independently C₁-C₁₀ alkyl, in another embodiment, -CH₃.

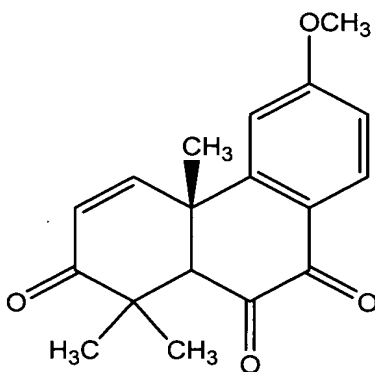
In another embodiment, R₇ is C₁-C₁₀ alkyl, in another embodiment -CH₃.

In another embodiment, the Diterpenoid Compounds of Formula (III) are in isolated and purified form.

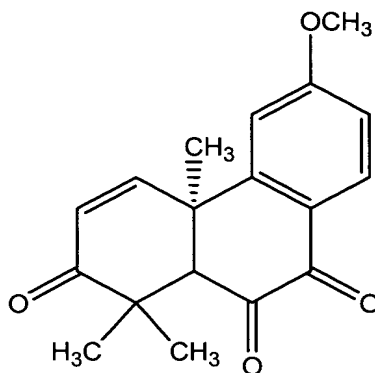
An illustrative Diterpenoid Compound of Formula (III) is:



70 ;



70a ;



70b

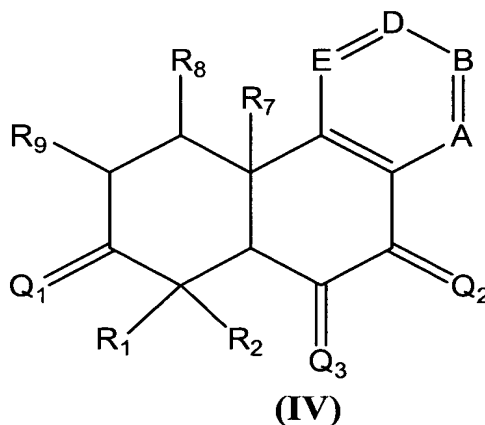
;

and pharmaceutically acceptable salts thereof.

It is to be understood that Compound **70** is the racemate of its corresponding enantiomers Compound **70a** and **70b**;

5.5 FORMULA IV

As stated above, the present invention encompasses novel compounds having the Formula (IV):



and pharmaceutically acceptable salts thereof, wherein Q₁ - Q₃, R₁ - R₉, A-E and halogen are defined above for Formula (IV).

The Diterpenoid Compounds of Formula (IV) are useful for treating or preventing cancer or neoplastic disease in a patient in need of such treatment or prevention. The Diterpenoid Compounds of Formula (IV) are also useful for inhibiting the growth of a cancer cell or neoplastic cell. The Diterpenoid Compounds of Formula (IV) are also useful for inducing cytotoxicity, *e.g.*, through apoptosis, in a cancer cell or neoplastic cell. The

Diterpenoid Compounds of Formula (IV) are further useful for treating or preventing a fungal infection. The Diterpenoid Compounds of Formula (IV) also useful for inhibiting the growth of a fungus.

In one embodiment, the Diterpenoid Compounds of Formula (IV) are those wherein Q_1 , Q_2 and Q_3 are =O.

In another embodiment, the Diterpenoid Compounds of Formula (IV) are those wherein A, B, D and E are CR_3 , CR_4 , CR_5 and CR_6 , respectively.

In another embodiment, the Diterpenoid Compounds of Formula (IV) are those wherein A is CR_3 , E is CR_6 , and R_3 and R_6 are hydrogen.

In another embodiment, the Diterpenoid Compounds of Formula (IV) are those wherein D is CR_5 and R_5 is C_1 - C_6 alkoxy, in another embodiment, $-OCH_3$.

In another embodiment, the Diterpenoid Compounds of Formula (IV) are those wherein B is CR_4 and R_4 is hydrogen.

In another embodiment, the Diterpenoid Compounds of Formula (IV) are those wherein B is CR_4 , R_4 is hydrogen, D is CR_5 and R_5 is C_1 - C_6 alkoxy.

In another embodiment, the Diterpenoid Compounds of Formula (IV) are those wherein B is CR_4 , R_4 is hydrogen, D is CR_5 and R_5 is $-OCH_3$.

In another embodiment, the Diterpenoid Compounds of Formula (IV) are those wherein B is CR_4 and R_4 is C_1 - C_{10} alkyl, in another embodiment, $-CH_3$.

In another embodiment, the Diterpenoid Compounds of Formula (IV) are those wherein B is CR_4 and R_4 is C_1 - C_{10} (hydroxy)alkyl, in another embodiment, hydroxymethyl.

In another embodiment, R_8 and R_9 are hydrogen.

In another embodiment, R_1 and R_2 are independently C_1 - C_{10} alkyl, in another embodiment, $-CH_3$.

In another embodiment, R_7 is C_1 - C_{10} alkyl, in another embodiment $-CH_3$.

In another embodiment, the Diterpenoid Compounds of Formula (IV) are in isolated and purified form.

5.6 METHODS FOR MAKING DITERPENOID COMPOUNDS

The Diterpenoid Compounds can be obtained using conventional organic synthesis or by using the following illustrative methods shown in Schemes 1-7 below.

Diterpenoid Compounds of Formula (I) or (II) can be obtained from a tetralone-type precursor such as depicted by compounds **13** in Scheme 1. Nucleophilic addition of R₇ using an appropriate organometallic reagent, such as a Grignard reagent (E.C. Ashby et al., *J. Am. Chem. Soc.*, 89:1964 (1967)), followed by dehydration (C. Utermohlen et al., *J. Org. Chem.*, 52:5574 (1987)) provides compounds **14**, which can undergo Diels-Alder cycloadditions with dienes such as compounds **15** (S. Danishefsky et al., *J. Am. Chem. Soc.*, 101:7001 (1979)), with or without Lewis acid catalysis, to yield after desilylation tricyclic intermediates **16**. The a, b- unsaturation of compounds **17** can be introduced by treating compounds **16** with a strong base such as lithium diisopropyl amide, followed by treatment with phenylselenium chloride, hydrogen peroxide and meta-chloroperoxybenzoic acid (M. Tius et al., *J. Am. Chem. Soc.*, 114:5959 (1992)). Compounds **17** can then be oxidized with, for example, chromium trioxide/sulphuric acid or IBX in DMSO and oxygen with potassium t-butoxide in t-butanol to provide compounds **18** (Nicolaou et al., *J. Am. Chem. Soc.* 123:3183 (2001); Nicolaou et al., *Angew. Chem. Int. Ed.* 40:207 (2001)), which are in equilibrium with enols **19**. Compounds **18a**, **18b**, **19a** and **19b** can be further modified to provide thioketones, imines, hydrazones, hydrazines, and arylhydrazones, as structurally described in Formulas (I), (II), (III) and (IV) by using simple organic transformations well recognised by chemists skilled in the art of organic synthesis.

Scheme 2 illustrates a synthesis of Diterpenoid Compounds of subgeneric formulas **5a**, **5b**, **6a**, and **6b**. This route uses compounds **21**, which can be obtained (Scheme 3) in three steps from IBX-DMSO oxidation of aryl- or heteroaryl- substituted propanols **26** (Nicolaou et al., *J. Am. Chem. Soc.* 123:3183 (2001)), followed by Wittig reaction with aldehydes **27** (B. Maryanoff et al., *J. Am. Chem. Soc.*, 107:217,(1985); A. Maercker, *Organic Reactions*, 14:270 (1965)), desilylation of resultant product **28** using a reagent such as, but not limited to, TBAF, and bromination using tribromophosphine in a solvent such as dichloromethane to provide bromide **21**.

Carbonyl-containing compounds **20** can be coupled with intermediates **21** using reagents such as NaH or n-butyllithium in tetrahydrofuran to provide compounds **22** (S. Welch et al., *J. Am. Chem. Soc.*, 101:6768 (1979)), which can then be cyclised to the tricyclic intermediates **23** using manganese acetate in acetic acid (B. Snider et al., *J. Org. Chem.*, 50:3659 (1985)). The a, b- unsaturation of compounds **24** can be introduced by treating compounds **23** with lithium diisopropyl amide, phenylselenium chloride, hydrogen peroxide and meta-chloroperoxybenzoic acid (M. Tius et al., *J. Am. Chem. Soc.*, 114:5959 (1992)).

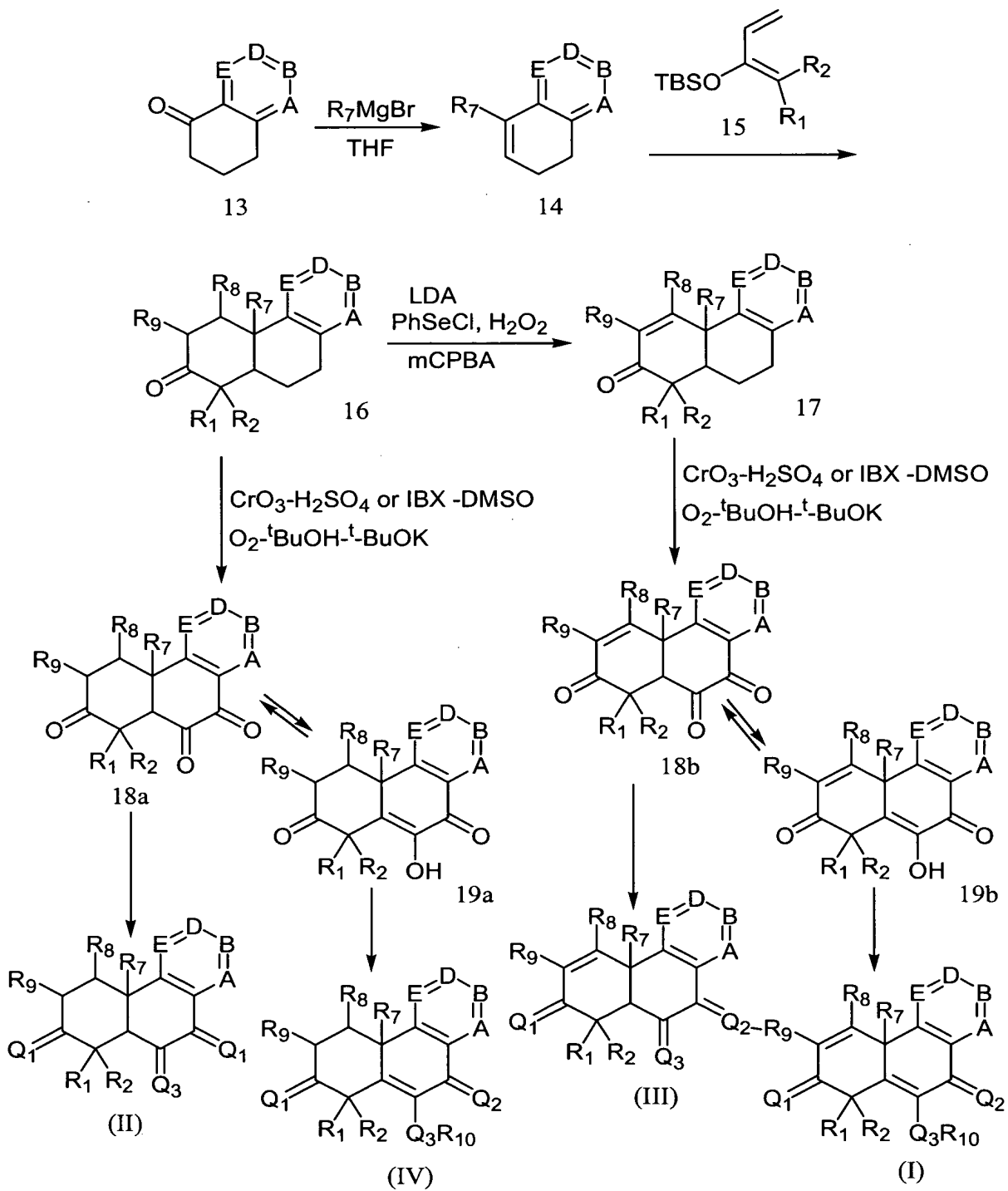
Oxidation of compounds **23** and **24** with, for example, chromium trioxide/acetic acid or oxygen and potassium t-butoxide in t-butanol, provides compounds **6a** and **6b**, respectively, which are in equilibrium with the enol forms of compounds **25a** and **25b**, respectively. Compounds **5a** and **5b** can be prepared from compounds **25a** and **25b**, respectively, by O-alkylating or O-acylating using well-known synthetic methods.

12-Methoxypodocarpa-8,11,13-trieneoic acid (**29**) is a useful starting material for Diterpenoid Compounds **11a**, **11b**, **12a**, and **12b** (Schemes 4 and 5). With reference to Scheme 4, compounds **30** can be obtained by treating compound **29** with a strong base such as n-butyllithium followed by alkylation using an alkyl halide, aldehyde, ketone, ester or epoxide. The resulting compounds **30** can then be treated with lead tetraacetate and monoperphthalic acid to provide epoxides **31** (R. Cambie and T. Fullerton, *Aust. J. Chem.*, 24:2611 (1971)), which can then be treated with lithium diethylamide and n-lithioethelenediamine to yield the tricyclic compounds **32** (R. Cambie and T. Fullerton, *Aust. J. Chem.*, 24:2611 (1971)). Compounds **33** can then be obtained by oxidizing compounds **32** with a reagent such as chromium trioxide and sulphuric acid, forming an enolate from the resultant ketone using a basic solution such as potassium t-butoxide in t-butanol and quenching the enolate with an alkylating agent, such as methyl iodide (B. Snider et al., *J. Org. Chem.*, 50:3659 (1985)). Reduction of compounds **33** with a metal such as palladium in a solvent/acid mixture such as ethanol and acetic acid provides the tricyclic ketones **34** (H. Thompson et al., *J. Org. Chem.*, 41:2903 (1976)). Compounds such as **11a**, where $R_5 = \text{OMe}$, can be obtained by treating compounds **34** with lithium diisopropyl amide, followed by phenylselenium chloride, hydrogen peroxide and meta-chloroperoxybenzoic acid, further followed by oxidation using, for example, chromium trioxide/acetic acid or oxygen with potassium t-butoxide in t-butanol (M. Tius et al., *J. Am. Chem. Soc.*, 114:5959 (1992)). Oxidation of compounds **34** using chromium trioxide/acetic acid and oxygen with potassium t-butoxide in t-butanol provides **11b** where $R_5 = \text{OCH}_3$. Compounds **11a** and **11b** ($R_5 = \text{OMe}$) are in equilibrium with triketo compounds **12a** and **12b**, respectively.

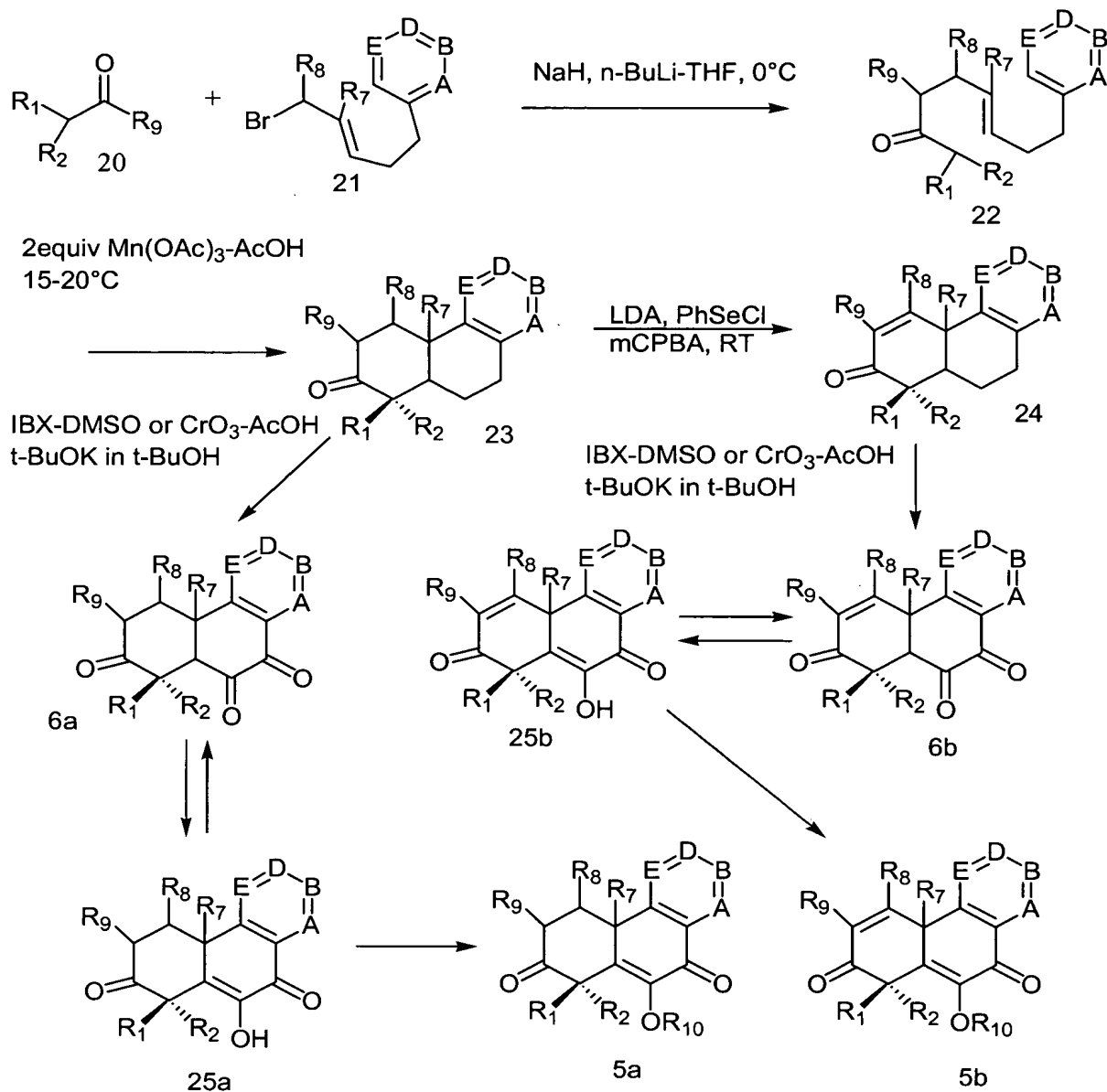
Scheme 5 depicts an alternative method useful for the preparation of Diterpenoid Compounds **11a**, **11b**, **12a**, and **12b**. For example, compounds **35** having R_5 functional groups other than $-\text{OCH}_3$ can be obtained by demethylating compound **29** with a reagent such as boron trichloride, treating the resultant phenoxide with triflic anhydride or any other suitable triflating agent, and then displacing the triflate with an organometallic reagent such as a boronate or a cuprate. The conversion of compounds **35** to Diterpenoid Compounds **11a**,

11b, **12a**, and **12b** can be achieved using the same or similar synthetic methods as described in Scheme 4 for converting compounds **29** to those of formula **11a**, **11b** ($R_5=OMe$) and **12a**, **12b** ($R_5=OMe$)(R. Cambie and T. Fullerton, *Aust. J. Chem.*, 24:2611 (1971); M. Tius et al., *J. Am. Chem. Soc.*, 114:5959 (1992)).

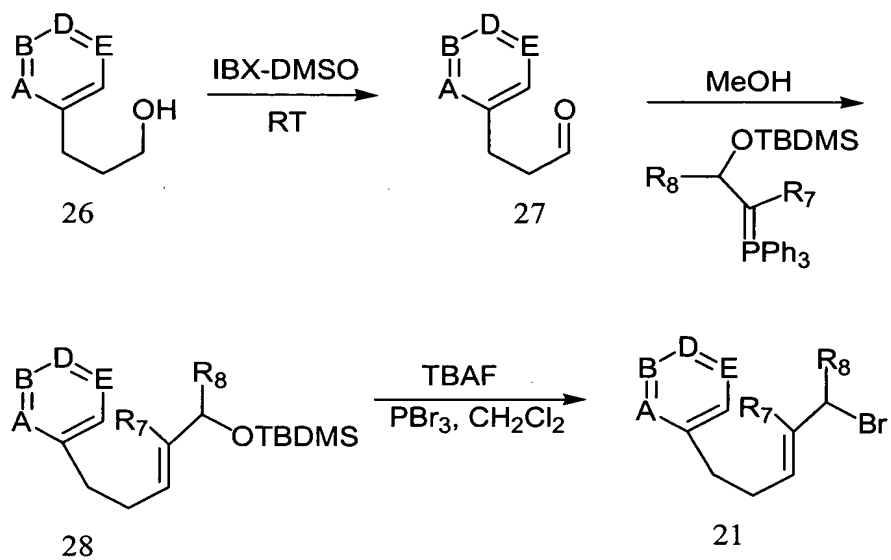
Scheme 1



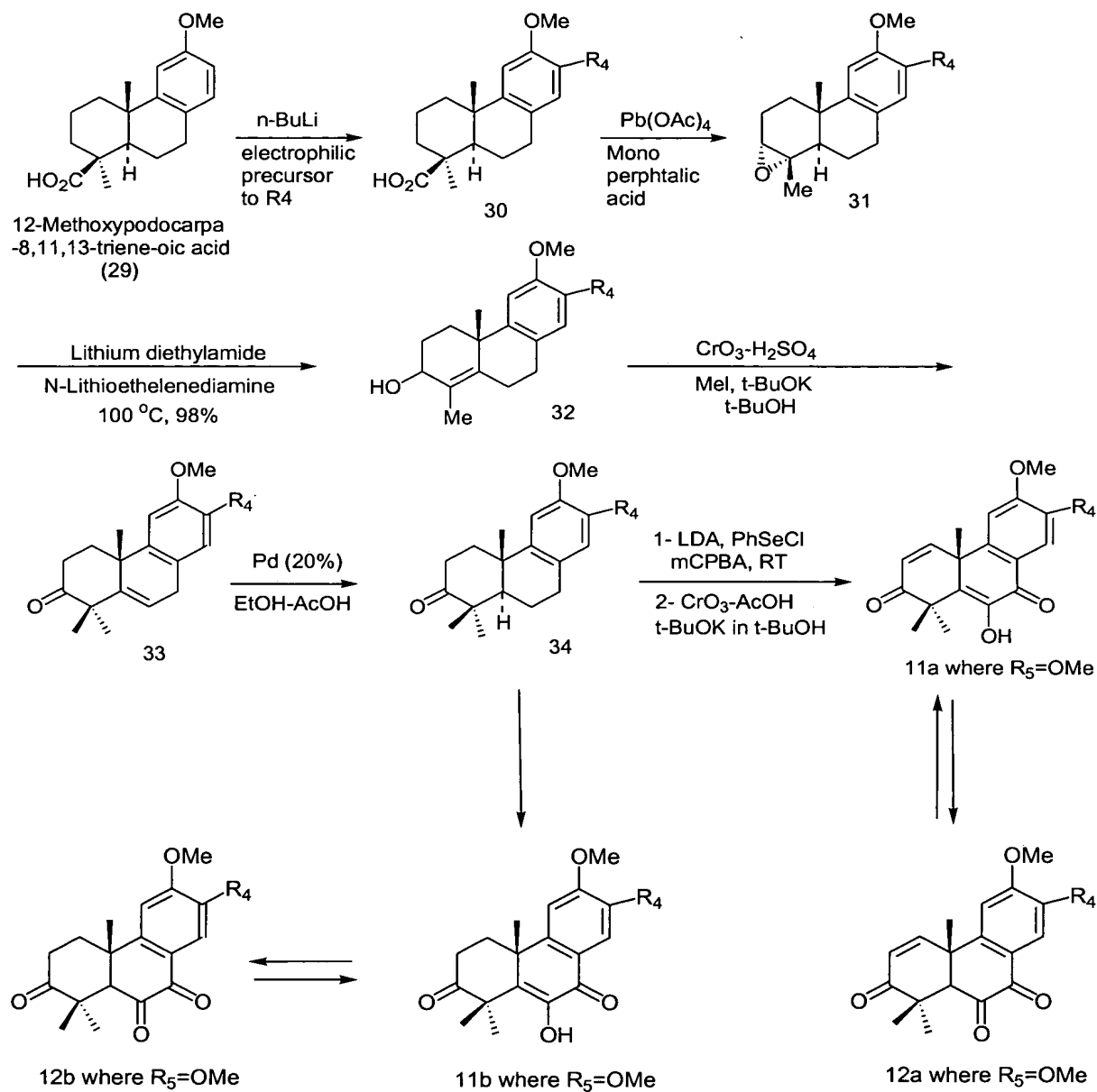
Scheme 2



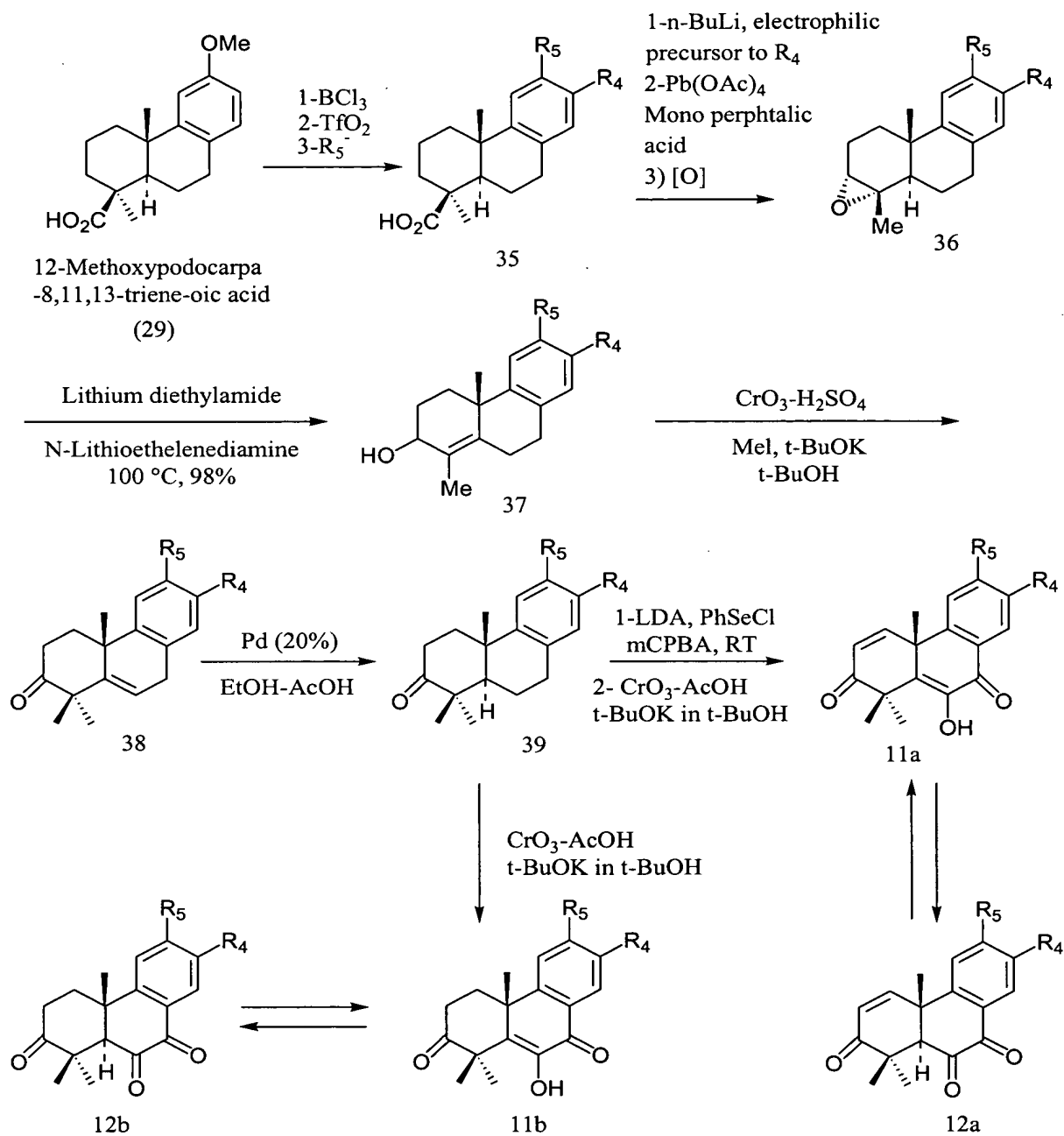
Scheme 3



Scheme 4



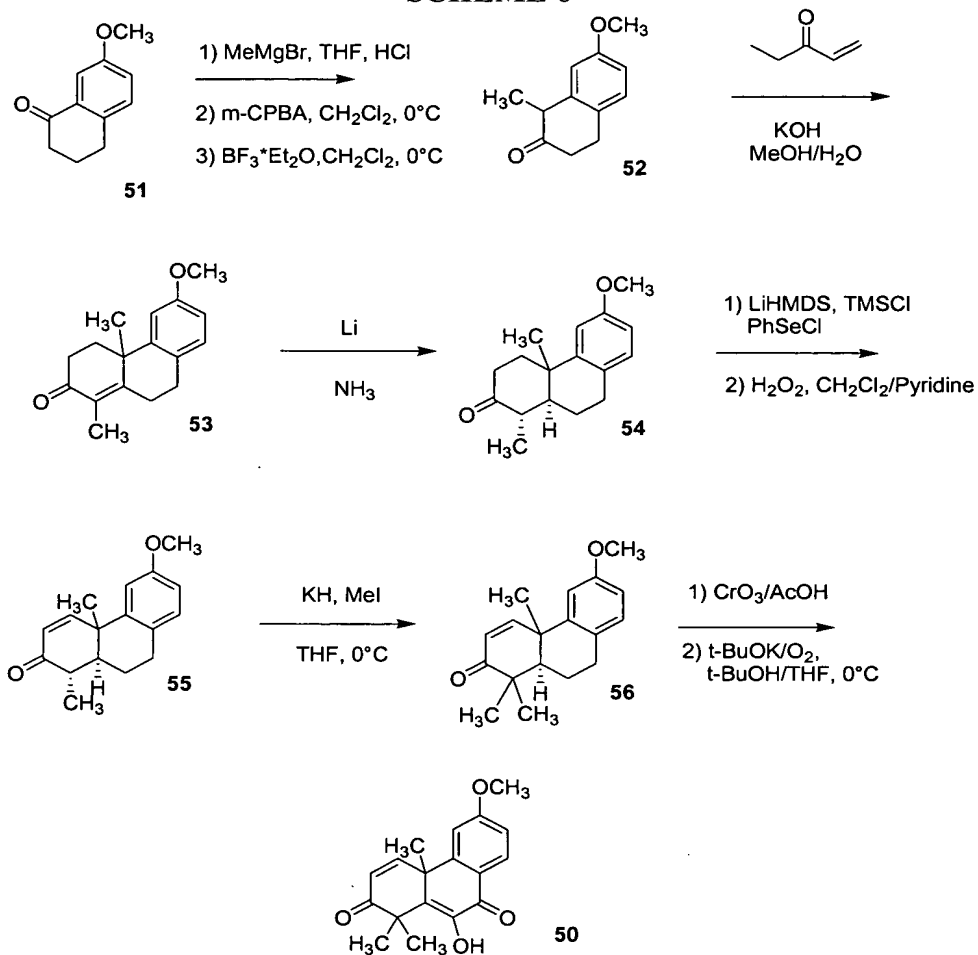
Scheme 5



Synthesis of Compound 50

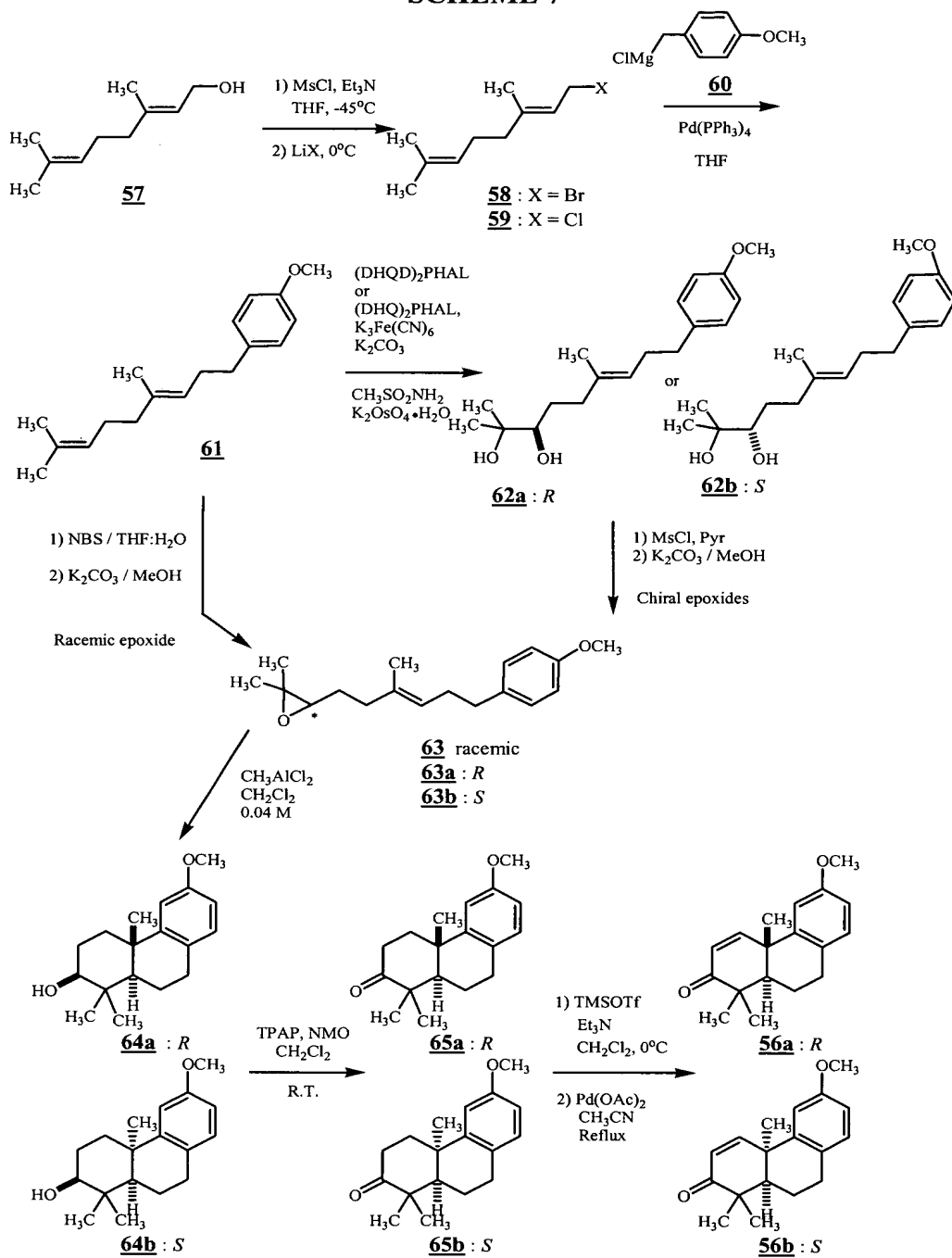
Compound **50** can be synthesized as illustrated in Scheme 6.

SCHEME 6

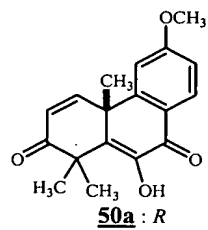


Compound **50a** and Compound **50b** can be synthesized as illustrated in Scheme 7.

SCHEME 7



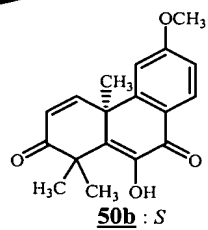
56a



1) CrO₃/AcOH

2) *t*-BuOK/O₂,
t-BuOH/THF, 0°C

56b



5.7 THERAPEUTIC/PROPHYLACTIC ADMINISTRATION AND COMPOSITIONS

Due to their activity, the Diterpenoid Compounds are advantageously useful in veterinary and human medicine. For example, the Diterpenoid Compounds are useful for treating or preventing cancer or neoplastic disease, inhibiting the growth of a cancer cell or neoplastic cell, inducing cytotoxicity, *e.g.*, through apoptosis, in a cancer cell or neoplastic cell, treating or preventing a fungal infection, or inhibiting the growth of a fungus.

When administered to a patient, *e.g.*, an animal for veterinary use or to a human for clinical use, or when made to contact a cell or tissue, the Diterpenoid Compounds can be in isolated and purified form.

The present compositions, which comprise a Diterpenoid Compound, can be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa) and can be administered together with another active agent. Administration can be systemic or local. Various delivery systems are known, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, capsules, etc., and can be used to administer a Diterpenoid Compound. In certain embodiments, more than one Diterpenoid Compound is administered to a patient. Methods of administration include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, oral, sublingual, intranasal, intracerebral, intravaginal, transdermal, rectally, by inhalation, or topically to the ears, nose, eyes, or skin. The mode of administration can be left to the discretion of the practitioner, and can depend in-part upon the site of the medical condition (such as the site of cancer or neoplastic disease or fungal infection).

In specific embodiments, it might be desirable to administer one or more Diterpenoid Compounds locally to the area in need of treatment. This can be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, *e.g.*, in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, by convection or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a cancer, tumor or neoplastic or pre-neoplastic tissue or fungal infection.

In certain embodiments, it might be desirable to administer one or more Diterpenoid Compounds by any suitable route, including intraventricular and intrathecal injection. Intraventricular injection can be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir.

Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent, or via perfusion in a fluorocarbon or synthetic pulmonary surfactant. In certain embodiments, the Diterpenoid Compounds can be formulated as a suppository, with traditional binders and carriers such as triglycerides.

In another embodiment, the Diterpenoid Compounds can be delivered in a vesicle, in particular a liposome (*see* Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; *see generally ibid.*)

In yet another embodiment, the Diterpenoid Compounds can be delivered in a controlled-release system. In one embodiment, a pump can be used (*see* Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (*see* *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); *see also* Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled-release system can be placed in proximity of the target of the Diterpenoid Compounds, thus requiring only a fraction of the systemic dose (*see, e.g.*, Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)). Other controlled-release systems discussed in the review by Langer (*Science* 249:1527-1533 (1990)) can be used.

The present compositions comprise an effective amount of a Diterpenoid Compound, which can be in isolated and purified form, together with a suitable amount of a pharmaceutically acceptable carrier so as to provide a useful form for administration to the patient.

In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in

humans. The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which a Diterpenoid Compound is administered. Such pharmaceutical carriers can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical carriers can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents can be used. When administered to a patient, the Diterpenoid Compounds and pharmaceutically acceptable carriers can be sterile. Water is a useful carrier when the Diterpenoid Compound is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The present compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

The present compositions can take the form of solutions, suspensions, emulsion, tablets, pills, pellets, capsules, capsules containing liquids, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, or any other form suitable for use. In one embodiment, the pharmaceutically acceptable carrier is a capsule (see *e.g.*, U.S. Patent No. 5,698,155). Other examples of suitable pharmaceutical carriers are described in “Remington’s Pharmaceutical Sciences” by E.W. Martin.

In one embodiment, the Diterpenoid Compounds are formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, Diterpenoid Compounds intended for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the compositions can also include a solubilizing agent. Compositions for intravenous administration can optionally include a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the Diterpenoid Compound is to be administered by infusion, it can be dispensed, for example, with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the Diterpenoid Compound is administered by injection, an ampoule of sterile water

for injection or saline can be provided so that the ingredients can be mixed prior to administration.

Compositions for oral delivery can be in the form of tablets, lozenges, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups, or elixirs, for example. Orally administered compositions can contain one or more optionally agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry; coloring agents; and preserving agents, to provide a pharmaceutically palatable preparation. Moreover, where in tablet or pill form, the compositions can be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving compound are also suitable for orally administered Diterpenoid Compounds. In these later platforms, fluid from the environment surrounding the capsule is imbibed by the driving compound, which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero order delivery profile as opposed to the spiked profiles of immediate release formulations. A time delay material such as glycerol monostearate or glycerol stearate can also be used. Oral compositions can include standard carriers such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, or magnesium carbonate. Such carriers can be of pharmaceutical grade.

The effective amount of the Diterpenoid Compound depends on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays can optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the compositions will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable effective amounts for intravenous administration generally range from about 10 micrograms to about 1 gram per kilogram body weight, in one embodiment from about 20 micrograms to about 500 micrograms, about 400 micrograms to about 2 milligrams, about 1 milligram to about 5 milligram, about 2 milligram to about 20 milligram, about 10 milligram to about 60 milligram, about 50 milligram to about 200 milligram, about 100 milligram to about 500 milligram, or about 200 milligram to about 800 milligram of Diterpenoid Compound per kilogram body weight. In specific embodiments of the invention, the effective amount for an i.v. dose ranges from about 10 to about 40, about 40 to about 60,

about 60 to about 100, or about 100 to about 200 micrograms per kilogram body weight. In other embodiments, the effective amount for an i.v. dose ranges from about 75 to about 150, about 150 to about 250, about 250 to about 375 or about 375 to about 500 or about 400 to about 800 micrograms per kilogram body weight. In specific embodiments of the invention, the effective amount for an i.v. dose ranges from about 0.5 to about 2, from about 1 to about 10, from about 10 to about 40, about 40 to about 60, about 60 to about 100, or about 100 to about 200 milligrams per kilogram body weight. In other embodiments, the effective amount for an i.v. dose ranges from about 75 to about 150, about 150 to about 250, about 250 to about 375 or about 375 to about 500 milligrams per kilogram body weight. Suitable effective amounts for intranasal administration generally range from about 0.01 pg/kg body weight to about 1 mg/kg, from about 0.5 mg/kg to about 800 mg/kg body weight. Suppositories generally contain an effective amount in the range of about 0.5% to about 10% by weight. Oral compositions can contain from about 10% to about 95% of Diterpenoid Compound. In specific embodiments of the invention, suitable effective amounts for oral administration generally range from about 0.1 micrograms to about 10 milligrams, from about 0.75 micrograms to about 1 milligram, from about 1 to about 500 micrograms, from about 200 micrograms to about 2 milligrams, from about 1 milligram to about 10 milligram, from about 5 milligram to about 50 milligram, from about 20 milligram to about 200 milligram, or from about 100 milligram to about 800 milligram of Diterpenoid Compound per kilogram body weight. In specific embodiments, the effective amount for an oral dose ranges from about 1 to about 10, about 10 to about 30, about 30 to about 90, or about 90 to about 150 micrograms per kilogram body weight. In other embodiments, the oral dose ranges from about 150 to about 250, about 250 to about 325, about 325 to about 450 or about 450 to about 1000 micrograms per kilogram body weight. In other embodiments, the oral dose ranges from about 150 to about 250, about 250 to about 325, about 325 to about 450 or about 450 to about 1000 milligrams per kilogram body weight. Effective amounts can be extrapolated from dose-response curves derived from *in vitro* or animal model test systems. Such animal models and systems are well known in the art. In one embodiment, for testing the effectiveness of a Diterpenoid Compound in an *in vitro* cell culture, concentrations from about 0.1 micromolar to about 10 micromolar, from about 0.2 micromolar to about 10 micromolar, from about 0.5 micromolar to about 5 micromolar, or from about 0.2 micromolar to about 5 micromolar can be used.

The invention also provides pharmaceutical packs or kits comprising one or more containers containing one or more Diterpenoid Compounds. Optionally associated with such container(s) can be instructions for use of one or more Diterpenoid Compounds or a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In one embodiment, *e.g.*, when administered for the treatment or prevention of cancer, the kit can also contain one or more chemotherapeutic agents useful for treating cancer or a neoplastic disease to be administered prior to, subsequent to, or in combination with a Diterpenoid Compound. In another embodiment, *e.g.*, when administered for the treatment or prevention of a fungal infection, the kit can also contain one or more other anti-fungal agents to be administered prior to, subsequent to or in combination with a Diterpenoid Compound. Such other anti-fungal agents include, but are not limited to, ketoconazole, itraconazole, amphotericin B, polyoxines, nikkomycines, carboxyamides, aromatic carbohydrates, carboxines, morpholines, inhibitors of sterol biosynthesis, and organophosphorus compounds.

The Diterpenoid Compounds can be assayed *in vitro*, and then *in vivo*, for the desired therapeutic or prophylactic activity, prior to use in humans. For example, *in vitro* assays can be used to determine whether administration of a specific Diterpenoid Compound or combination of Diterpenoid Compounds is preferred.

In one embodiment, a patient-tissue sample is grown in culture, and contacted or otherwise administered with a Diterpenoid Compound, and the effect of the Diterpenoid Compound upon the tissue sample is observed and compared with a non-contacted tissue. In other embodiments, a cell culture model is used in which the cells of the cell culture are contacted or otherwise administered with a Diterpenoid Compound, and the effect of the Diterpenoid Compound upon the tissue sample is observed and compared with a non-contacted cell culture. Generally, a lower level of proliferation or survival of the contacted cells compared to the non-contacted cells indicates that the Diterpenoid Compound is effective to treat or prevent cancer or a neoplastic disease. The Diterpenoid Compounds can also be demonstrated to be effective and safe using animal model systems.

In one embodiment, a fungus sample from an infected patient is grown in culture and contacted or otherwise administered with a Diterpenoid Compound, and the effect of the Diterpenoid Compound upon the growth of the fungus is observed and compared with a non-contacted tissue. Generally, a lower level of proliferation or survival of the contacted fungus

compared to the non-contracted fungus indicates that the Diterpenoid Compound is effective to treat or prevent the fungal infection. The Diterpenoid Compounds can also be demonstrated to be effective and safe using animal model systems.

Other methods will be known to the skilled artisan and are within the scope of the invention.

5.8 INHIBITION OF CANCER AND NEOPLASTIC CELLS AND DISEASE

The Diterpenoid Compounds can be shown to inhibit tumor cell proliferation, cell transformation or tumorigenesis *in vitro* and *in vivo* using a variety of assays known in the art, or described herein. Such assays may use cells of a cancer cell line, or cells from a patient. Many assays well-known in the art can be used to assess such survival and/or growth; for example, cell proliferation can be assayed by measuring (³H)-thymidine incorporation, by direct cell count, by detecting changes in transcription, translation or activity of known genes such as proto-oncogenes (*e.g.*, *fos*, *myc*) or cell cycle markers (Rb, cdc2, cyclin A, D1, D2, D3, E, etc). The levels of such protein and mRNA and activity can be determined by any method well known in the art. For example, protein can be quantitated by known immunodiagnostic methods such as Western blotting or immunoprecipitation using commercially available antibodies (for example, many cell cycle marker antibodies are from Santa Cruz Inc.). mRNA can be quantitated using methods that are well known and routine in the art, for example, using northern analysis, RNase protection, the polymerase chain reaction in connection with the reverse transcription. Cell viability can be assessed by using trypan-blue staining or other cell death or viability markers known in the art. In a specific embodiment, the level of cellular ATP is measured to determine cell viability. Differentiation can be assessed, for example, visually based on changes in morphology.

The present invention provides for cell cycle and cell proliferation analysis using a variety of techniques known in the art, including but not limited to the following:

As one example, bromodeoxyuridine (BRDU) incorporation can be used as an assay to identify proliferating cells. The BRDU assay identifies a cell population undergoing DNA synthesis by incorporation of BRDU into newly synthesized DNA. Newly synthesized DNA can then be detected using an anti-BRDU antibody (*see* Hoshino et al., 1986, Int. J. Cancer 38, 369; Campana et al., 1988, J. Immunol. Meth. 107, 79).

Cell proliferation can also be examined using (^3H)-thymidine incorporation (*see e.g.*, Chen, J., 1996, *Oncogene* 13:1395-403; Jeoung, J., 1995, *J. Biol. Chem.* 270:18367-73). This assay allows for quantitative characterization of S-phase DNA synthesis. In this assay, cells synthesizing DNA will incorporate (^3H)-thymidine into newly synthesized DNA. Incorporation can then be measured by standard techniques in the art such as by counting of radioisotope in a Scintillation counter (*e.g.* Beckman LS 3800 Liquid Scintillation Counter).

Detection of proliferating cell nuclear antigen (PCNA) can also be used to measure cell proliferation. PCNA is a 36 kilodalton protein whose expression is elevated in proliferating cells, particularly in early G1 and S phases of the cell cycle and therefore can serve as a marker for proliferating cells. Positive cells can be identified by immunostaining using an anti-PCNA antibody (*see* Li et al., 1996, *Curr. Biol.* 6:189-199; Vassilev et al., 1995, *J. Cell Sci.* 108:1205-15).

Cell proliferation can be measured by counting samples of a cell population over time (*e.g.* daily cell counts). Cells can be counted using a hemacytometer and light microscopy (*e.g.* HyLite hemacytometer, Hausser Scientific). Cell number can be plotted against time in order to obtain a growth curve for the population of interest. In one embodiment, cells counted by this method are first mixed with the dye Trypan-blue (Sigma), such that living cells exclude the dye, and are counted as viable members of the population.

DNA content and/or mitotic index of the cells can be measured, for example, based on the DNA ploidy value of the cell. For example, cells in the G1 phase of the cell cycle generally contain a 2N DNA ploidy value. Cells in which DNA has been replicated but have not progressed through mitosis (*e.g.* cells in S-phase) exhibit a ploidy value higher than 2N and up to 4N DNA content. Ploidy value and cell-cycle kinetics can be further measured using propidium iodide assay (*see e.g.* Turner, T., et al., 1998, *Prostate* 34:175-81). Alternatively, the DNA ploidy can be determined by quantitation of DNA Feulgen staining (which binds to DNA in a stoichiometric manner) on a computerized microdensitometry staining system (*see e.g.*, Bacus, S., 1989, *Am. J. Pathol.* 135:783-92). In another embodiment, DNA content can be analyzed by preparation of a chromosomal spread (Zabalou, S., 1994, *Hereditas* 120:127-40; Pardue, 1994, *Meth. Cell Biol.* 44:333-351).

The expression of cell-cycle proteins (*e.g.*, CycA, CycB, CycE, CycD, cdc2, Cdk4/6, Rb, p21, and p27) provide information relating to the proliferative state of a cell or population of cells. For example, identification in an anti-proliferation signaling pathway can

be indicated by the induction of p21^{cip1}. Increased levels of p21 expression in cells result in delayed entry into G1 of the cell cycle (Harper et al., 1993, Cell 75:805-816; Li et al., 1996, Curr. Biol. 6:189-199). p21 induction can be identified by immunostaining using a specific anti-p21 antibody available commercially (e.g. Santa Cruz). Similarly, cell-cycle proteins can be examined by Western blot analysis using commercially available antibodies. In another embodiment, cell populations are synchronized prior to detection of a cell cycle protein. Cell cycle proteins can also be detected by FACS (fluorescence-activated cell sorter) analysis using antibodies against the protein of interest.

Detection of changes in length of the cell cycle or speed of cell cycle can also be used to measure inhibition of cell proliferation by the Diterpenoid Compounds. In one embodiment the length of the cell cycle is determined by the doubling time of a population of cells (e.g., using cells contacted or not contacted with one or more Diterpenoid Compounds). In another embodiment, FACS analysis is used to analyze the phase of cell cycle progression, or purify G1, S, and G2/M fractions (*see e.g.*, Delia, D. et al., 1997, Oncogene 14:2137-47).

Lapse of cell cycle checkpoint(s), and/or induction of cell cycle checkpoint(s), can be examined using the methods described herein, or by any method known in the art. Without limitation, a cell cycle checkpoint is a mechanism that ensures that the different steps of cell division occur in a particular order. Checkpoint genes are defined by mutations that allow late events to occur without prior completion of an early event (Weinert, T., and Hartwell, L., 1993, Genetics, 134:63-80). Induction or inhibition of cell cycle checkpoint genes can be assayed, for example, by Western blot analysis, or by immunostaining, for example. Lapse of cell cycle checkpoints can be further assessed by the progression of a cell through the checkpoint without prior occurrence of specific events (e.g. progression into mitosis without complete replication of the genomic DNA).

In addition to the effects of expression of a particular cell cycle protein, activity and post-translational modifications of proteins involved in the cell cycle can play an integral role in the regulation and proliferative state of a cell. The invention provides for assays involved in detecting post-translational modifications (e.g. phosphorylation) by any method known in the art. For example, antibodies that detect phosphorylated tyrosine residues are commercially available, and can be used in Western blot analysis to detect proteins with such modifications. In another example, modifications such as myristylation, can be detected on thin layer chromatography or reverse phase h.p.l.c. (*see e.g.*, Glover, C., 1988, Biochem. J. 250:485-91; Paige, L., 1988, Biochem J.;250:485-91).

Activity of signaling and cell cycle proteins and/or protein complexes is often mediated by a kinase activity. The present invention provides for analysis of kinase activity by assays such as the histone H1 assay (*see e.g.*, Delia, D. et al., 1997, *Oncogene* 14:2137-47).

The Diterpenoid Compounds can also be demonstrated to alter cell proliferation in cultured cells *in vitro* using methods which are well known in the art. Specific examples of cell culture models include, but are not limited to, for lung cancer, primary rat lung tumor cells (Swafford et al., 1997, *Mol. Cell. Biol.*, 17:1366-1374) and large-cell undifferentiated cancer cell lines (Mabry et al., 1991, *Cancer Cells*, 3:53-58); colorectal cell lines for colon cancer (Park and Gazdar, 1996, *J. Cell Biochem. Suppl.* 24:131-141); multiple established cell lines for breast cancer (Hambly et al., 1997, *Breast Cancer Res. Treat.* 43:247-258; Gierthy et al., 1997, *Chemosphere* 34:1495-1505; Prasad and Church, 1997, *Biochem. Biophys. Res. Commun.* 232:14-19); a number of well-characterized cell models for prostate cancer (Webber et al., 1996, *Prostate*, Part 1, 29:386-394; Part 2, 30:58-64; and Part 3, 30:136-142; Boulikas, 1997, *Anticancer Res.* 17:1471-1505); for genitourinary cancers, continuous human bladder cancer cell lines (Ribeiro et al., 1997, *Int. J. Radiat. Biol.* 72:11-20); organ cultures of transitional cell carcinomas (Booth et al., 1997, *Lab Invest.* 76:843-857) and rat progression models (Vet et al., 1997, *Biochim. Biophys Acta* 1360:39-44); and established cell lines for leukemias and lymphomas (Drexler, 1994, *Leuk. Res.* 18:919-927, Tohyama, 1997, *Int. J. Hematol.* 65:309-317).

The Diterpenoid Compounds can also be demonstrated to inhibit cell transformation (or progression to malignant phenotype) *in vitro*. In this embodiment, cells with a transformed cell phenotype are contacted with one or more Diterpenoid Compounds, and examined for change in characteristics associated with a transformed phenotype (a set of *in vitro* characteristics associated with a tumorigenic ability *in vivo*), for example, but not limited to, colony formation in soft agar, a more rounded cell morphology, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, release of proteases such as plasminogen activator, increased sugar transport, decreased serum requirement, or expression of fetal antigens, etc. (*see* Luria et al., 1978, *General Virology*, 3d Ed., John Wiley & Sons, New York, pp. 436-446).

Loss of invasiveness or decreased adhesion can also be used to demonstrate the anti-cancer effects of the Diterpenoid Compounds. For example, an aspect of the formation of a metastatic cancer is the ability of a precancerous or cancerous cell to detach from primary site

of disease and establish a novel colony of growth at a secondary site. The ability of a cell to invade peripheral sites reflects its potential for a cancerous state. Loss of invasiveness can be measured by a variety of techniques known in the art including, for example, induction of E-cadherin-mediated cell-cell adhesion. Such E-cadherin-mediated adhesion can result in phenotypic reversion and loss of invasiveness (Hordijk et al., 1997, Science 278:1464-66).

Loss of invasiveness can further be examined by inhibition of cell migration. A variety of 2-dimensional and 3-dimensional cellular matrices are commercially available (Calbiochem-Novabiochem Corp. San Diego, CA). Cell migration across or into a matrix can be examined using microscopy, time-lapsed photography or videography, or by any method in the art allowing measurement of cellular migration. In a related embodiment, loss of invasiveness is examined by response to hepatocyte growth factor (HGF). HGF-induced cell scattering is correlated with invasiveness of cells such as Madin-Darby canine kidney (MDCK) cells. This assay identifies a cell population that has lost cell scattering activity in response to HGF (Hordijk et al., 1997, Science 278:1464-66).

Alternatively, loss of invasiveness can be measured by cell migration through a chemotaxis chamber (Neuroprobe/ Precision Biochemicals Inc. Vancouver, BC). In such assay, a chemo-attractant agent is incubated on one side of the chamber (*e.g.*, the bottom chamber) and cells are plated on a filter separating the opposite side (*e.g.*, the top chamber). In order for cells to pass from the top chamber to the bottom chamber, the cells must actively migrate through small pores in the filter. Checkerboard analysis of the number of cells that have migrated can then be correlated with invasiveness (*see e.g.*, Ohnishi, T., 1993, Biochem. Biophys. Res. Commun. 193:518-25).

The Diterpenoid Compounds can also be demonstrated to inhibit tumor formation *in vivo*. A vast number of animal models of hyperproliferative disorders, including tumorigenesis and metastatic spread, are known in the art (see Table 317-1, Chapter 317, "Principals of Neoplasia," in *Harrison's Principals of Internal Medicine, 13th Edition*, Isselbacher et al., eds., McGraw-Hill, New York, p. 1814, and Lovejoy et al., 1997, J. Pathol. 181:130-135). Specific examples include for lung cancer, transplantation of tumor nodules into rats (Wang et al., 1997, Ann. Thorac. Surg. 64:216-219) or establishment of lung cancer metastases in SCID mice depleted of NK cells (Yono and Sone, 1997, Gan To Kagaku Ryoho 24:489-494); for colon cancer, colon cancer transplantation of human colon cancer cells into nude mice (Gutman and Fidler, 1995, World J. Surg. 19:226-234), the cotton top tamarin model of human ulcerative colitis (Warren, 1996, Aliment. Pharmacol. Ther. 10

Supp 12:45-47) and mouse models with mutations of the adenomatous polyposis tumor suppressor (Polakis, 1997, Biochim. Biophys. Acta 1332:F127-F147); for breast cancer, transgenic models of breast cancer (Dankort and Muller, 1996, Cancer Treat. Res. 83:71-88; Amundadittir et al., 1996, Breast Cancer Res. Treat. 39:119-135) and chemical induction of tumors in rats (Russo and Russo, 1996, Breast Cancer Res. Treat. 39:7-20); for prostate cancer, chemically-induced and transgenic rodent models, and human xenograft models (Royai et al., 1996, Semin. Oncol. 23:35-40); for genitourinary cancers, induced bladder neoplasm in rats and mice (Oyasu, 1995, Food Chem. Toxicol 33:747-755) and xenografts of human transitional cell carcinomas into nude rats (Jarrett et al., 1995, J. Endourol. 9:1-7); and for hematopoietic cancers, transplanted allogeneic marrow in animals (Appelbaum, 1997, Leukemia 11 (Suppl. 4):S15-S17). Further, general animal models applicable to many types of cancer have been described, including, but not restricted to, the p53-deficient mouse model (Donehower, 1996, Semin. Cancer Biol. 7:269-278), the Min mouse (Shoemaker et al., 1997, Biochem. Biophys. Acta, 1332:F25-F48), and immune responses to tumors in rat (Frey, 1997, Methods, 12:173-188).

For example, a Diterpenoid Compound can be administered to a test animal, preferably a test animal predisposed to develop a type of tumor, and the test animal subsequently examined for a decreased incidence of tumor formation in comparison with controls not administered the Diterpenoid Compound. Alternatively, a Diterpenoid Compound can be administered to a test animal having a tumor (*e.g.*, animals in which tumors have been induced by introduction of malignant, neoplastic, or transformed cells, or by administration of a carcinogen), and the tumors in the test animals can be subsequently examined for tumor regression and compared with controls that were not administered with the Diterpenoid Compound.

The Diterpenoid Compounds are useful for inhibiting the growth of a cancer cell or neoplastic cell and for inducing cytotoxicity, *e.g.*, through apoptosis, of a cancer cell or neoplastic cell *in vivo*. Inhibiting the growth of a cancer cell or neoplastic cell and inducing cytotoxicity, *e.g.*, through apoptosis, in a cancer cell or neoplastic cell *in vivo* is useful for treating, preventing and inhibiting the growth of a cancer. The Diterpenoid Compounds are useful for inhibiting the growth of a cancer cell or neoplastic cell and for inducing cytotoxicity, *e.g.*, through apoptosis, in a cancer cell or neoplastic cell *in vitro*. Inhibiting the growth of a cancer cell or neoplastic cell and inducing cytotoxicity, *e.g.*, through apoptosis, in

a cancer cell or neoplastic cell *in vitro* is useful for assays to determine optimal concentration ranges of effectiveness of a Diterpenoid Compound.

5.8.1 INDUCING APOPTOSIS IN A CANCER CELL OR A NEOPLASTIC CELL

Without being bound by theory, apoptosis is a morphologically and biochemically distinct form of cell death that occurs in response to a diverse range of stimuli, including irradiation and activation of death receptors such as Fas and the tumor necrosis factor receptor. Neoplastic transformation or cancerous growth of a cell can trigger apoptosis of that cell. Impaired apoptosis is therefore a significant factor in the aetiology of cancer and neoplastic diseases.

Morphologic criteria that can be used to describe apoptotic cells include condensation and margination of chromatin, cytoplasmic vacuolization, cellular shrinkage, increase in cellular density, nuclear fragmentation and apoptotic body formation.

Without being bound by theory, Applicants believe that the Diterpenoid Compounds induce apoptosis in a cancer cell or in a neoplastic cell. Moreover, without being bound by theory, Applicants believe that Diterpenoid Compounds induce apoptosis selectively in a cancer cell or in a neoplastic cell, relative to a non-cancer cell or non-neoplastic cell. In one embodiment, a Diterpenoid Compound induces apoptosis with at least 2-fold selectivity in a cancer cell or in a neoplastic cell, relative to a non-cancer cell or non-neoplastic cell. In certain embodiments, a Diterpenoid Compound induces apoptosis with at least 5-fold, 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, 75-fold, 100-fold, 150-fold, 200-fold or 250-fold selectivity in a cancer cell or in a neoplastic cell, relative to a non-cancer cell or non-neoplastic cell. In certain embodiments, a Diterpenoid Compound induces apoptosis with at most 5-fold, 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, 75-fold, 100-fold, 150-fold, 200-fold or 250-fold selectivity in a cancer cell and/or in a neoplastic cell, relative to a non-cancer cell or non-neoplastic cell. When selectivity in a cancer cell or neoplastic cell is n-fold, relative to a non-cancer or non-neoplastic cell, a Diterpenoid Compound induces apoptosis in n-times as many cancer cells or neoplastic cells than non-cancer cells or non-neoplastic cells.

Without being bound by theory, inducing apoptosis selectively in cancer cells or in neoplastic cells is useful for treating cancer or a neoplastic disease in a patient.

5.8.2 TREATMENT OR PREVENTION OF CANCER OR A NEOPLASTIC DISEASE IN COMBINATION WITH CHEMOTHERAPY OR RADIOTHERAPY

Cancer or a neoplastic disease, including, but not limited to, neoplasms, tumors, metastases, or any disease or disorder characterized by uncontrolled cell growth, can be treated or prevented by administration of an effective amount of a Diterpenoid Compound.

In certain embodiments, the present methods for treating or preventing cancer or a neoplastic disease comprise administering an effective amount of a Diterpenoid Compound and another active agent, such as a chemotherapeutic or anti-cancer agent, including, but not limited to, methotrexate, taxol, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, Cisplatin, carboplatin, mitomycin, dacarbazine, procarbazine, etoposides, campathecins, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, asparaginase, vinblastine, vincristine, vinorelbine, paclitaxel, and docetaxel. In another embodiment, the other chemotherapeutic or anti-cancer agent includes, but is not limited to, those listed in Table 1.

TABLE 1 CHEMOTHERAPEUTICS AND OTHER ANTI-CANCER AGENTS

<u>Radiation:</u>	γ -radiation
<u>Alkylating agents</u>	
Nitrogen mustards:	cyclophosphamide Ifosfamide trofosfamide Chlorambucil
Nitrosoureas:	carmustine (BCNU) Lomustine (CCNU)
Alkylsulphonates	busulfan Treosulfan
Triazenes:	Dacarbazine
Platinum containing compounds:	Cisplatin carboplatin
<u>Plant Alkaloids</u>	
Vinca alkaloids:	vincristine Vinblastine Vindesine

Taxoids: Vinorelbine
paclitaxel
Docetaxol

DNA Topoisomerase Inhibitors

Epipodophyllins: etoposide
Teniposide
Topotecan
9-aminocamptothecin
campto irinotecan
crisnatol

mytomycins:

mytomycin C Mytomycin C

Anti-metabolites

Anti-folates:

DHFR inhibitors: methotrexate
Trimetrexate

IMP dehydrogenase Inhibitors: mycophenolic acid
Tiazofurin
Ribavirin
EICAR

Ribonucleotide reductase Inhibitors: hydroxyurea
deferrioxamine

Pyrimidine analogs:

Uracil analogs 5-Fluorouracil

Floxuridine
Doxifluridine
Ratitrexed

Cytosine analogs cytarabine (ara C)
Cytosine arabinoside
fludarabine

Purine analogs: mercaptopurine
Thioguanine

Hormonal therapies:

Receptor antagonists:

Anti-estrogens Tamoxifen

Raloxifene
megestrol
LHRH agonists: goserelin
Leuprolide acetate
Anti-androgens: flutamide
bicalutamide

Retinoids/Retinoids

Vitamin D3 analogs: EB 1089
CB 1093
KH 1060

Photodynamic therapies: vertoporphin (BPD-MA)
Phthalocyanine
photosensitizer Pc4
Demethoxy-hypocrellin A
(2BA-2-DMHA)

Cytokines: Interferon- α
Interferon- γ
Tumor necrosis factor

Others:

Isoprenylation inhibitors: Lovastatin
Dopaminergic neurotoxins: 1-methyl-4-phenylpyridinium ion
Cell cycle inhibitors: staurosporine
Kinase inhibitors: Imatinib

Actinomycins: Actinomycin D
Dactinomycin

Bleomycins: bleomycin A2
Bleomycin B2
Peplomycin

Anthracyclines: daunorubicin
Doxorubicin (adriamycin)
Idarubicin
Epirubicin
Pirarubicin

Zorubicin
Mitoxantrone

MDR inhibitors: verapamil

Ca²⁺ ATPase inhibitors: thapsigargin

In other embodiments, the methods for treating or preventing cancer or a neoplastic disease comprise administering an effective amount of a Diterpenoid Compound and an effective amount of radiation therapy or another chemotherapeutic agent, in one embodiment, with a chemotherapeutic agent with which treatment of the cancer has not been found to be refractory. The Diterpenoid Compound can be administered to a patient that has also undergone surgery as treatment for the cancer.

In another specific embodiment, the invention provides methods for treating or preventing cancer that has shown to be refractory to treatment with a chemotherapy and/or radiation therapy.

In a specific embodiment, a Diterpenoid Compound is administered concurrently with chemotherapy or radiation therapy. In another specific embodiment, chemotherapy or radiation therapy is administered prior or subsequent to administration of a Diterpenoid Compound, preferably at least an hour, five hours, 12 hours, a day, a week, a month, more preferably several months (*e.g.*, up to three months), subsequent to administration of the Diterpenoid Compound.

The chemotherapy or radiation therapy administered concurrently with, or prior or subsequent to, the administration of a Diterpenoid Compound can be accomplished using any method known in the art. The chemotherapeutic agents can be administered in a series of sessions, any one or a combination of the chemotherapeutic agents listed above can be administered. With respect to radiation therapy, any radiation therapy protocol can be used depending upon the type of cancer to be treated or prevented. For example, but not by way of limitation, x-ray radiation can be administered; in particular, high-energy megavoltage (radiation of greater than 1 MeV energy) can be used for deep tumors, and electron beam and orthovoltage x-ray radiation can be used for skin cancers. Gamma-ray emitting radioisotopes, such as radioactive isotopes of radium, cobalt and other elements, can also be administered to expose tissues to radiation.

Additionally, the invention provides methods for treating or preventing cancer or neoplastic disease with a Diterpenoid Compound as an alternative to chemotherapy or

radiation therapy where the chemotherapy or the radiation therapy has proven or might prove too toxic, *e.g.*, results in unacceptable or unbearable side effects, for the patient being treated. The patient being treated with the Diterpenoid Compound can, optionally, be treated with other cancer treatments such as surgery, radiation therapy or chemotherapy, depending on which treatment is found to be acceptable or bearable.

5.8.3 CANCER AND NEOPLASTIC DISEASE TREATABLE OR PREVENTABLE

Cancers or neoplastic diseases and related disorders that can be treated or prevented by administration of an effective amount of a Diterpenoid Compound and cancer cells and neoplastic cells whose growth can be inhibited or in which cytotoxicity, *e.g.*, through apoptosis, can be induced by contacting with an effective amount of a Diterpenoid Compound include but are not limited to those listed in Table 2 (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia):

TABLE 2
CANCERS AND NEOPLASTIC DISORDERS

Leukemia
acute leukemia
acute lymphocytic leukemia
acute myelocytic leukemia
myeloblastic
promyelocytic
myelomonocytic
monocytic
erythroleukemia
chronic leukemia
chronic myelocytic (granulocytic) leukemia
chronic lymphocytic leukemia
Polycythemia vera
Lymphoma
Hodgkin's disease
non-Hodgkin's disease
Multiple myeloma
Waldenström's macroglobulinemia
Heavy chain disease
Solid tumors
sarcomas and carcinomas
fibrosarcoma
myxosarcoma
liposarcoma

chondrosarcoma
osteogenic sarcoma
chordoma
angiosarcoma
endotheliosarcoma
lymphangiosarcoma
lymphangioendotheliosarcoma
synovioma
mesothelioma
Ewing's tumor
leiomyosarcoma
rhabdomyosarcoma
colon carcinoma
pancreatic cancer
breast cancer
ovarian cancer
prostate cancer
squamous cell carcinoma
basal cell carcinoma
adenocarcinoma
sweat gland carcinoma
sebaceous gland carcinoma
papillary carcinoma
papillary adenocarcinomas
cystadenocarcinoma
medullary carcinoma
bronchogenic carcinoma
renal cell carcinoma
hepatoma
bile duct carcinoma
choriocarcinoma
seminoma
embryonal carcinoma
Wilms' tumor
cervical cancer
uterine cancer
testicular tumor
lung carcinoma
small cell lung carcinoma
bladder carcinoma
epithelial carcinoma
glioma
astrocytoma
medulloblastoma
craniopharyngioma
ependymoma
pinealoma
hemangioblastoma
acoustic neuroma

oligodendroglioma
meningioma
melanoma
neuroblastoma
retinoblastoma
NSCL-LC carcinoma
NSCL-adrenocarcinoma
Liver cancer
Breast epithelial cancer
Endothelial cancer
Bronchial epithelial cancer

In specific embodiments, cancer, malignancy or dysproliferative changes (such as metaplasias and dysplasias), or hyperproliferative disorders, are treated or prevented in the ovary, breast, colon, lung, skin, pancreas, prostate, bladder, cervix or uterus. In other specific embodiments, sarcoma, melanoma, or leukemia is treated or prevented.

In one embodiment, the Diterpenoid Compounds are useful for treating or preventing cancers including prostate cancer, such as hormone-insensitive prostate cancer, Neuroblastoma, Lymphoma (preferably follicular or Diffuse Large B-cell), Breast (for example Estrogen- receptor positive), Colorectal, Endometrial, Ovarian, Lymphoma (for example non-Hodgkin's), Lung (for example Small cell), or Testicular (for example germ cell).

In another embodiment, the Diterpenoid Compounds are useful for inhibiting the growth of a cell derived from a cancer or neoplasm such as prostate (in one embodiment, hormone- insensitive), Neuroblastoma, Lymphoma (in one embodiment, follicular or Diffuse Large B-cell), Breast (in one embodiment, Estrogen-receptor positive), Colorectal, Endometrial, Ovarian, Lymphoma (in one embodiment, non-Hodgkin's), Lung (in one embodiment, Small cell), or Testicular (in one embodiment, germ cell).

In other embodiments of the invention, the Diterpenoid Compounds are useful for inhibiting the growth of a cell, said cell being derived from a cancer or neoplasm in Table 2 or herein.

5.9 INHIBITION OF FUNGAL GROWTH AND TREATMENT AND PREVENTION OF FUNGAL INFECTIONS

The invention provides methods for treating or preventing a fungal infection, comprising administering to a patient in need of such treatment or prevention an effective

amount of a Diterpenoid Compound. Fungal Infections that can be treated or prevented by administering an effective amount of a Diterpenoid Compound include, but are not limited to, Candida (including *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. stellatoidea*, *C. krusei*, *C. parakrusei*, *C. lusitanae*, *C. pseudotropicalis*, *C. guilliermondi*, *C. dubliniensis*, *C. famata* or *C. glabrata*), Aspergillus (including *A. fumigatus*, *A. flavus*, *A. niger*, *A. nidulans*, *A. terreus*, *A. sydowi*, *A. flavatus* or *A. glaucus*), Cryptococcus, Histoplasma, Coccidioides, Paracoccidioides, Blastomyces, Basidiobolus, Conidiobolus, Rhizopus, Rhizomucor, Mucor, Asbidia, Mortierella, Cunninghamella, Saksenaea, Pseudallescheria, Paecilomyces, Fusarium, Trichophyton, Trichosporon Microsporum, Epidermophyton, Scytalidium, Malassezia, Actinomycetes, Sporothrix, Penicillium, Sacharomyces, Pneumocystis or Scopulariopsis infections.

In certain embodiments, such fungal infections in animals, including humans, can be a systemic, topical or mucosal infection.

In view of their antifungal activity, Diterpenoid Compounds are useful in the treatment of variety of fungal infections in animals, including humans. Such infections can be superficial, cutaneous, subcutaneous or systemic mycotic infections such as respiratory tract infections, gastrointestinal infections, cardiovascular infections, urinary tract infections, CNS infections, candidiasis and chronic mucocandidiasis and skin infections caused by fungi, cutaneous and mucocutaneous candidiasis, athletes foot, paronychia, fungal nappy rash, candida vulvitis, candida balanitis and otitis externa. They may also be used as prophylactic agents to prevent systemic and topical fungal infections. Use as prophylactic agents may be appropriate as part of a selective gut decontamination regimen in the prevention of infection in immunocomprised patients, e.g., AIDS patients and patients receiving transplant therapy.

The invention further provides a method for inhibiting the growth of a fungus comprising contacting the fungus with an effective amount of a Diterpenoid Compound. The fungi whose growth can be inhibited with a Diterpenoid Compound include Candida (including *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. stellatoidea*, *C. krusei*, *C. parakrusei*, *C. lusitanae*, *C. pseudotropicalis*, *C. guilliermondi*, *C. dubliniensis*, *C. famata* or *C. glabrata*), Aspergillus (including *A. fumigatus*, *A. flavus*, *A. niger*, *A. nidulans*, *A. terreus*, *A. sydowi*, *A. flavatus* or *A. glaucus*), Cryptococcus, Histoplasma, Coccidioides, Paracoccidioides, Blastomyces, Basidiobolus, Conidiobolus, Rhizopus, Rhizomucor, Mucor, Asbidia,

Mortierella, Cunninghamella, Saksenaea, Pseudallescheria, Paecilomyces, Fusarium, Trichophyton, Trichosporon Microsporum, Epidermophyton, Scytalidium, Malassezia, Actinomycetes, Sporothrix, Penicillium, Sacharomyces, Pneumocystis or Scopulariopsis.

In certain embodiments, the Diterpenoid Compounds can be used as anti-fungal agents *in vitro* or *in vivo*. In a specific embodiment, the Diterpenoid Compounds can be used to prevent growth of a fungus wherever absence of fungal growth is desired, such as on or in food, medical instruments or devices, clothing, furniture and home appliances.

The following examples exemplify non-limiting aspects of the present invention.

6. EXAMPLES

6.1 ESTABLISHMENT AND MANIPULATION OF A PLANT CELL SUSPENSION CULTURE OF *LINUM ARBOREUM*

A plant cell culture of *Linum arboreum* (Linaceae) was prepared using shoots of *L. arboreum*. The shoots were sterilized by immersion for 1 minute in 70% ethanol followed by immersion for 25 minutes in a solution of sodium hypochlorite (concentration 15%). The sterile shoots were chopped into small pieces of approximately 5 mm and placed upon solidified callus induction medium B5 (Gamborgs B5 recipe (Exp. Cell. Res. **50**: 148 (1968)) containing 2,4-dichlorophenoxyacetic acid (2,4-D) (1 mg/L), kinetin (0.1 mg/L), sucrose (2%)). Callus initiations were incubated in continuous low light at 23°C. Upon establishment of callus, the material was used to initiate suspension cultures.

To establish suspension cultures, portions of established callus were placed in 100 mL conical flasks containing liquid medium B88, modified after Gamborgs B5 recipe to contain 2,4-D (1 mg/L), kinetin (0.1 mg/L), coconut water (10%) + 3% sucrose. The liquid medium was replenished at 14 day intervals. After 4 months the established suspension culture was routinely maintained in a 250 mL conical flask, by transferring 40 mL of a 14 or 21 day old suspension culture into 100 mL fresh B88 medium. The culture was incubated at 25°C in continuous low light and shaken at 140 rpm.

Accumulation of isolated compounds was induced in the *Linum arboreum* suspension culture according to either one of the following protocols.

(1) 2L conical flasks each containing 750 mL of a secondary metabolite production medium B49 (Gamborgs B5, 5% sucrose, no hormones), were each inoculated with 260 mL

of a 14-day old suspension culture grown on B88 medium. The cultures were incubated under low light conditions (approximately 30 lux) at 25°C for 28 days.

(2) A 40 mL aliquot of a day 0 suspension growing on B88 medium was transferred to a 100 mL flask. On day 3, a sterile solution of 5-azacytidine (5-AC) in water was added for a final concentration of 3×10^{-5} M, and the resultant mixture was incubated for 11 days. At this point the 40mL 5-AC-treated culture was subcultured, twice before inoculating 190mL B49 production medium in a 500mL flask with 70mL of a 14 day-old suspension. The culture was incubated under low light conditions (approximately 30 lux) at 25°C for 7 days following inoculation, filter-sterilized methyl jasmonate (250 uM final concentration) and an autoclaved *Candida albicans* preparation (50 mg/L final concentration) were added. The *C. albicans* preparation was obtained by growing a culture of strain ATCC28516 on YEPD media (yeast extract 1%, yeast peptone 2%, glucose 2%) to maximum cell density and twice autoclaving the total yeast culture prior to addition to plant cultures. The culture was incubated for a further 7 days before harvest of biomass.

6.2 ISOLATION OF COMPOUNDS 48 AND 49

At harvest the plant cell culture of *Linum arboreum*, the culture was centrifuged and the supernatant liquid was decanted. The residual biomass was freeze-dried, and subsequently extracted. Biomass from the culture method (1) in Example 6.1 was used in the following isolation procedure.

81.85g of dried biomass corresponding to a 6L culture of *Linum arboreum* obtained above were placed in two 3L glass beakers, 2L of methanol was added to each beaker and stirred at room temperature for 20 hours. The mixture was then filtered under vacuum through a fritted funnel. After filtration the marc was washed twice with 250 mL portions of methanol and then the washings were combined with the main filtrate. The combined filtrate and washings were evaporated to dryness under vacuum using a rotary evaporator. The yield of the methanol extract (E11) was 22.8 g (see Fig 1A).

The E11 extract was dissolved in 2L of water and then partitioned with 2 x 1L of butanol. The butanol phases were separated, combined and then concentrated to dryness. The yield of the butanol exact (E11B) was 7.65 g (33.5% from the methanol extract).

150 mL of HP-20 were placed in a 360 mL fritted glass column (3.8 x 30 cm) and equilibrated with 10% acetonitrile. Approximately 7.5g of the E11B extract were re-dissolved in methanol and absorbed into 70 mL of HP-20. The absorbed HP-20 extract was

placed on top of the HP-20 column and eluted with an aqueous acetonitrile gradient followed by methanol and acetone. The active fraction (A4) was eluted with methanol. It yielded approximately 420mg (1.8% from the methanol extract).

Approximately 1 mg of the A4 fraction was fractionated by HPLC using the Gem5.met method (see Experimental Methods below). Fractions were collected in a 96-well plates.

The remainder of the A4 fraction was triturated with 2x50 mL of hexane under mechanical stirring. After centrifugation, the soluble material was concentrated to dryness yielding 197 mg of a hexane fraction (B1). The hexane insoluble material was triturated with 2 x 50 mL of ethyl acetate. After centrifugation, the soluble material was concentrated to dryness yielding 40 mg of an ethyl acetate fraction (B2). The ethyl acetate insoluble material yielded 135 mg of a methanol soluble fraction (B3). All fractions, B1-B3 were analyzed by HPLC.

B1 and B2 fractions were subjected to preparative reverse phase HPLC using the Gem8p.met method (see Experimental Methods below). Fractions B1C6 and B2C5 (peaks at R_t ~25.8 min) were combined to yield 4.3 mg of Compound **48**. Fraction B1C4 (peak at R_t ~14.3 min) yielded 1.5 mg of Compound **49**.

EXPERIMENTAL METHODS:

HPLC: Gem5.met

Column: Xterra™ RP₁₈ (150 x 7.8 mm, 7 μ)

Flow Rate: 3 mL/min

Detection: UV @ 205, 220 and 280 nm

Solvent system:

Time (min.)	H ₂ O (%)	ACN (%)
0.0	80	20
5.0	60	40
35.0	0	100
42.0	0	100
45.0	80	20
55.0	80	20

HPLC: Gem8n.met

Column: Xterra™ RP₁₈ (300 x 19 mm, 7μ)

Flow Rate: 14 mL/min

Detection: UV @ 205, 220 and 277 nm

Solvent system:

Time (min.)	H ₂ O (%)	ACN (%)
0.0	80	20
5.0	55	45
50.0	0	100
65.0	0	100
70.0	80	20
85.0	80	20

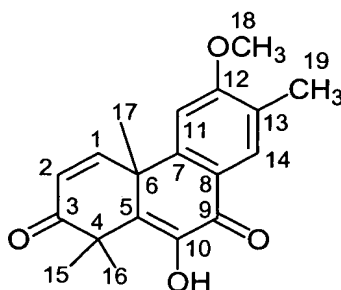
STRUCTURE ELUCIDATION:

Compound **48**'s ¹H-NMR and ¹³C-NMR assignments were made based on the analysis of their 2D-NMR experiments. The mass spectra showed a peak at m/z 313 corresponding to M+1 ion. The UV spectra showed a maximum absorption at λ 218, 238 (sh), 277 and 326 nm. The ¹H-NMR spectra run in CDCl₃ reveals the presence of four olefinic protons [δ 8.01 (s), δ 7.39 (d, J = 10 Hz), δ 6.94 (s), δ 6.29 (d, J = 10 Hz)], four methyl groups [δ 2.29 (s), δ 1.69 (s), δ 1.60 (s), δ 1.57 (s)], one methoxy group at δ 3.99 (s) and one exchangeable proton at δ 7.22 (s). The ¹³C-NMR spectra run in CDCl₃ showed signals for 19 carbons. Multiplicity information was obtained from the DEPT experiments. The olefinic proton at δ 7.39 COSY with the signal at δ 6.29. The magnitude of their coupling constant (J = 10 Hz) is in agreement with the cis configuration. 2D-NMR experiments including COSY, HMQC and HMBC were performed in order to assign all proton and carbon signals (Table 3). ¹H-¹³C correlations observed from the HMBC experiment are summarized in Fig 1B.

Compound **49**'s mass spectra showed a peak at m/z 329 corresponding to M+1 ion. The UV spectra showed a maximum absorption at λ 216, 238 (sh), 277 and 327 nm. The ¹H-NMR spectra run in CDCl₃ reveals the presence of four olefinic protons [δ 8.21 (s), δ 7.38 (d, J = 10 Hz), δ 7.02 (s), δ 6.30 (d, J = 10 Hz)], three methyl groups [δ 1.70 (s), δ 1.60 (s), δ 1.58 (s)], one methoxy group at δ 4.04 (s), one methylene group at δ 4.77 and one exchangeable proton at δ 7.21(s). The ¹³C-NMR spectra run in CDCl₃ showed signals for 19 carbons. Structure elucidation was performed by comparison of their ¹H and ¹³C-NMR

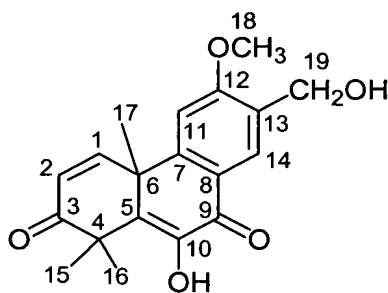
spectra with Compound **48**. The difference appears to be in the aromatic ring where a methylene group is replacing a methyl group. NMR assignments are summarized in Table 4.

Table 3. NMR Assignments of Compound **48**



Atom #	¹³ C-NMR (in CDCl ₃)	¹ H-NMR (in CDCl ₃)	¹ H-NMR (in MeOD)
1	150.6	7.39 (1H, d, <i>J</i> = 10Hz)	7.65 (1H, d, <i>J</i> = 10Hz)
2	128.4	6.29 (1H, d, <i>J</i> = 10Hz)	6.27 (1H, d, <i>J</i> = 10Hz)
3	202.7	----	----
4	48.1	----	----
5	133.1	----	----
6	41.7	----	----
7	148.8	----	----
8	120.8	----	----
9	179.0	----	----
10	142.9	----	----
11	105.7	6.94 (1H, s)	7.24 (1H, s)
12	162.6	----	----
13	127.4	----	----
14	129.3	8.01 (1H, s)	7.92 (3H, s)
15	26.3	1.60 (3H, s)	1.58(3H, s)
16	21.1	1.69 (3H, s)	1.67 (3H, s)
17	38.9	1.57 (3H, s)	1.56 (3H, s)
18	55.7	3.99 (3H, s)	4.02 (3H, s)
19	15.9	2.29 (3H, s)	2.26 (3H, s)
OH	----	7.22 (1H, s)	----

Table 4. NMR Assignments of Compound **49**



Atom #	¹³ C-NMR (in CDCl ₃)	¹ H-NMR (in CDCl ₃)	¹ H-NMR (in MeOD)
1	150.1	7.38 (1H, d, <i>J</i> = 10Hz)	7.67 (1H, d, <i>J</i> = 10 Hz)
2	128.6	6.30 (1H, d, <i>J</i> = 10Hz)	6.30 (1H, d, <i>J</i> = 10Hz)
3	202.5	----	----
4	48.1	----	----
5	133.3	----	----
6	41.9	----	----
7	150.5	----	----
8	121.3	----	----
9	178.8	----	----
10	142.9	----	----
11	106.3	7.02 (1H, s)	7.30 (1H, s)
12	161.8	----	----
13	129.8	----	----
14	127.7	8.21 (1H, s)	8.22 (1H, s)
15	26.3	1.60 (3H, s)	1.59 (3H, s)
16	21.1	1.70 (3H, s)	1.68 (3H, s)
17	38.9	1.58 (3H, s)	1.58 (3H, s)
18	55.9	4.04 (3H, s)	4.03 (3H, s)
19	61.0	4.77 (2H, s)	4.67 (2H, s)
10-OH	----	7.21 (1H, s)	----

6.3 SYNTHESIS OF COMPOUND 50

Compound **50** was synthesized as illustrated in Scheme 6 and summarized below.

Tetralone **51** was treated with methylmagnesium bromide, and the resulting alcohol was eliminated with hydrochloric acid to provide an alkene. The alkene was treated with *m*-chloroperbenzoic acid, and the resultant epoxide was treated with boron trifluoride to provide tetralone **52** (Murphy *et al. J. Org. Chem.*, **1960**, *25*, 1386; Kuehne. *J. Amer. Chem. Soc.*, **1961**, *83*, 1492; Taylor; Chiang. *Tetrahedron Lett.*, **1977**, 1827). A Robinson's annelation was performed on tetralone **52** using a modified method (Shishido, K. *et al. J. Org. Chem.*, **1994**, *59*, 406-414), and the resulting enone **53** was reduced by a Birch reduction to provide ketone **54**. Enone **55** was obtained using known methods (Grieco *et al., J. Org. Chem.*, **1998**, *63*, 5929-5936) followed by the methylation of the enone to provide Compound **56**. Chromium(VI)oxide was used for the benzylic oxidation according to Rutledge (Cambie *et al., P. S. Aust. J. Chem.*, **1998**, *51*, 931-940) to give ketone **56**. The potassium enolate of ketone **56** was oxidized with oxygen, and the resultant 1,2-diketone formed was enolized *in-situ* to provide Compound **50**.

Synthesis of Compound **52**

Methylmagnesium bromide (100 mL; 3M solution; 300 mmol) was added to a stirred solution of 7-methoxy-1-teralone **51** (25 g; 142 mmol) in THF (400 mL) at -78°C. The resultant mixture was heated to room temperature and stirred for three hours. The solution was cooled to 0°C and water (60 mL) was added dropwise, followed by a solution of hydrochloric acid (60 mL conc. HCl and 30 mL water). The resultant solution was stirred at room temperature for one hour and then extracted with ethyl acetate (3 × 200mL). The combined organic layers were washed with a saturated aqueous solution of sodium bicarbonate (100 mL), dried over sodium sulfate, decanted and concentrated *in vacuo*. The resultant residue was dissolved in dichloromethane (400 mL) and cooled to 0°C, and to it was added *m*-chloroperbenzoic acid (38 g; 77%; 170 mmol). The resultant mixture was stirred at 0°C for 30 min and then quenched with a saturated aqueous solution of sodium bicarbonate (100 mL) and a solution of saturated aqueous sodium thiosulfate (50 mL). The organic and aqueous layers were separated, and the aqueous layer was extracted with dichloromethane (2 × 100 mL). The combined organic layers were dried over sodium sulfate, decanted and concentrated *in vacuo* to a final volume of 400 mL. The solution was cooled to 0°C, and to it was added boron trifluoride diethyletherate (0.9 mL; 7.1 mmol). After 15 min at 0°C the reaction was quenched with a solution of saturated aqueous sodium bicarbonate (100 mL). The organic and aqueous layers were separated, and the aqueous layer was extracted with

dichloromethane (2 × 100 mL). The combined organic layers were dried over sodium sulfate, decanted and concentrated *in vacuo*. The resultant residue was purified via column chromatography over silica gel (10% ethyl acetate in hexane eluent) to provide Compound **52** (20 g, 74%) as a colorless oil.

NMR ¹H (300 MHz, CDCl₃): δ (ppm) 1.46 (d, *J* = 8 Hz, 3H), 2.53 (m, 2H), 3.0 (m, 2H), 3.48 (q, *J* = 8 Hz, 1H), 3.82 (s, 3H), 6.77 (m, 2H), 7.13 (d, *J* = 8 Hz, 1H).

Synthesis of Compound **53**

To a stirred solution of potassium hydroxide (1.27 g; 22.7 mmol) in methanol (22 mL) and water (2.5 mL) at -15°C, was added, dropwise, Compound **52** (3.6 g; 18.9 mmol) in methanol (5 mL). After 30 min at -15°C, ethyl vinyl ketone (1.87 mL; 18.9 mmol) was added. The resultant solution was stirred 1 h at -15°C, heated at room temperature for 1 h and finally heated to 50°C for 2 hrs. The methanol was removed *in vacuo* and the residue was dissolved in water (30 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic layers were dried over sodium sulfate, decanted and concentrated *in vacuo*. The resultant residue was purified via column chromatography over silica gel (10% ethyl acetate in hexane eluent) to provide Compound **53** (3.5 g, 72%) as a pale yellow solid.

NMR ¹H (300 MHz, CDCl₃): δ (ppm) 1.52 (s, 3H), 1.85 (s, 3H), 2.06 (m, 1H), 2.3-3.1 (m, 7H), 3.80 (s, 3H), 6.72 (dd, *J* = 9 Hz and 2 Hz, 1H), 6.84 (d, *J* = 2 Hz, 1H), 7.03 (d, *J* = 9 Hz, 1H).

Synthesis of Compound **54**

To a stirred solution of lithium (948 mg; 136 mmol) in liquid ammonia (75 mL) at -78°C, was added dropwise a solution of Compound **53** (3.5 g; 13.6 mmol) and *t*-butanol (1.3 mL; 13.6 mmol) in tetrahydrofuran (25 mL). After 1.5 hr at -78°C, isoprene (7 mL; 68 mmol) was added dropwise, and the reaction mixture was heated to room temperature to allow the ammonia to evaporate. A saturated aqueous solution of ammonium chloride (50 mL) was added to the residue, and the resultant mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over sodium sulfate, decanted and concentrated *in vacuo*. The resultant residue was purified via column chromatography over silica gel (10% ethyl acetate in hexane eluent) to provide Compound **54** (1.64 g, 66%) as a white solid.

NMR ^1H (300 MHz, CDCl_3): δ (ppm) 1.12 (d, $J = 7$ Hz, 3H), 1.38 (s, 3H), 1.63 (m, 2H), 1.86 (m, 1H), 1.97 (m, 1H), 2.40-2.69 (m, 4H), 2.86 (m, 2H), 3.79 (s, 3H), 6.71 (dd, $J = 9$ Hz and 2 Hz, 1H), 6.85 (d, $J = 2$ Hz, 1H), 7.00 (d, $J = 9$ Hz, 1H).

Synthesis of Compound 55

Compound **54** was converted to Compound **55** using known methods (Grieco, *et al.* *J. Org. Chem.*, **1998**, *63*, 5929-5936).

NMR ^1H (300 MHz, CDCl_3): δ (ppm) 1.23 (d, $J = 7$ Hz, 3H), 1.34 (s, 3H), 1.67 (m, 1H), 2.00 (m, 2H), 2.43 (m, 1H), 2.88 (m, 2H), 3.80 (s, 3H), 5.97 (d, $J = 10$ Hz, 1H), 6.71 (dd, $J = 9$ Hz and 2 Hz, 1H), 6.95 (d, $J = 2$ Hz, 1H), 7.00 (d, $J = 9$ Hz, 1H), 7.54 (d, $J = 10$ Hz, 1H).

Synthesis of Compound 56

To a stirred suspension of potassium hydride (492 mg; 30% dispersion in mineral oil; 3.69 mmol) in tetrahydrofuran (8 mL) at 0°C , was added dropwise a solution of Compound **55** (315 mg; 1.23 mmol) in tetrahydrofuran (9 mL). After 30 min at 0°C , freshly distilled iodomethane (0.765 mL; 12.3 mmol) was added. The reaction mixture was stirred at 0°C for 1 hr and then quenched with a solution of saturated aqueous ammonium chloride (10 mL). The organic and aqueous layers were separated, and the aqueous layer was extracted with ethyl acetate ($3 \times 20\text{mL}$). The combined organic layers were dried over sodium sulfate, decanted and concentrated *in vacuo*. The resultant residue was purified via column chromatography over silica gel (15% ethyl acetate in hexane eluent) to provide Compound **56** (240 mg, 54%).

NMR ^1H (300 MHz, CDCl_3): δ (ppm) 1.18 (s, 3H), 1.20 (s, 3H), 1.40 (s, 3H), 1.89 (m, 2H), 2.14 (dd, $J = 11$ Hz and 4 Hz, 1H), 2.88 (m, 2H), 3.79 (s, 3H), 6.00 (d, $J = 10$ Hz, 1H), 6.71 (dd, $J = 9$ Hz and 2 Hz, 1H), 6.91 (d, $J = 2$ Hz, 1H), 7.02 (d, $J = 9$ Hz, 1H), 7.53 (d, $J = 10$ Hz, 1H).

Synthesis of Compound 50

To a stirred solution of Compound **56** (240 mg; 0.89 mmol) in acetic acid (6 mL) at 0°C , was added, before freezing of the acetic acid, a solution of chromium(VI) oxide (222 mg; 2.23 mmol) in acetic acid/water (1.2 mL/0.3 mL) dropwise over 10 min. The reaction mixture was stirred at 0°C for 15 min, and then water (10 mL) was added. The reaction

mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic layers were washed with a solution of saturated aqueous sodium bicarbonate (10 mL), dried over sodium sulfate, filtered through a short pad of silica gel, washed with ethyl acetate (50 mL) and concentrated *in vacuo*. The resultant residue was dissolved in tetrahydrofuran (3 mL) and added dropwise to a stirred solution of potassium *t*-butoxide (300 mg; 2.67 mmol) in *t*-butanol (5.5 mL) and tetrahydrofuran (13 mL) at 0°C. The resultant solution was stirred at 0°C under oxygen atmosphere (balloon) for 1 h and then quenched with a solution of saturated aqueous ammonium chloride (10 mL). The resultant reaction mixture was extracted with ethyl acetate (3 × 20mL), and the combined organic layers were dried over sodium sulfate, filtered through a short pad of silica gel, washed with ethyl acetate (50 mL) and concentrated *in vacuo*. The resultant residue was triturated in cold ethanol (4 mL) and filtered to provide Compound **50** (130 mg, 49%) as a white solid.

NMR ¹H (300 MHz, CDCl₃): δ (ppm) 1.57 (s, 3H), 1.59 (s, 3H), 1.69 (s, 3H), 3.95 (s, 3H), 6.27 (d, *J* = 10 Hz, 1H), 7.00 (dd, *J* = 9 Hz and 2 Hz, 1H), 7.08 (d, *J* = 2 Hz, 1H), 7.20 (s, 1H), 7.37 (d, *J* = 10 Hz, 1H), 8.20 (d, *J* = 9 Hz, 1H).

NMR ¹³C (75 MHz, CDCl₃): δ (ppm) 202.6, 179.0, 164.2, 150.8, 150.6, 143.0, 133.6, 130.1, 128.6, 121.6, 113.8, 111.0, 56.0, 48.4, 42.1, 39.3, 26.7, 21.5.

MP: 167°C-168°C

SYNTHESIS OF COMPOUND 50a AND COMPOUND 50b

Compound **50a** and Compound **50b** were synthesized as illustrated in Scheme 7.

Synthesis of Compound 61

Compound **61** was synthesized as described in Takahashi *et al.*, *Syn. Lett.* **1999**, 5, 6444-646; Corey, *et al. J. Am. Chem Soc.* **1997**, 119, 9927-9928; and Rosales *et al. J. Org. Chem.* **2002**, 67, 1167-1170.

To a solution of geraniol **57** (100 mol%; 5.0 mmole; 868 μL) in dry THF at -45°C was added methanesulfonyl chloride (130 mol%; 6.5 mmole; 502 μL) and triethylamine (200 mol%; 10 mmole; 1.4 mL). The resultant solution was stirred at -45°C for 45 min, then warmed to 0°C, at which point lithium chloride (400 mol%, 20 mmole, 848 mg as a solid) or lithium bromide (400 mol%, 20 mmole, 1.737g as a solution in 8 mL of THF via cannula) was added. The resultant solution was stirred for 1 hr at 0°C. Water was added (50 mL), and the resultant mixture was extracted using hexane (2 × 30 mL). The combined organic

solution was successively washed with saturated NaHCO₃ and brine, dried over Na₂SO₄, filtered and evaporated to provide crude allylic halides **58** and **59**, which were used directly, without further purification, in the next step.

To a solution of tetrakis(triphenylphosphine)palladium(0) (5 mol%; 50 μ mol; 58 mg) in dry and freshly degassed THF was added allylic halide **58** or **59** (100 mol%; 1.0 mmole; **58**: 217 mg; **59**: 173 mg). The resulting solution was stirred at room temperature (20°C) for 5 min then cooled to 0°C. *p*-Methoxybenzyl magnesium chloride **60** (130 mol%; 1.3 mmole; 5.2 mL of a 0.25 M solution in THF) was added via syringe and the reaction mixture was stirred at room temperature (20°C, **58**: 5 hrs; **59**: 3 hrs). Water was added (20 mL), and the mixture was extracted using Et₂O (2 \times 10 mL). The combined organic solution was dried over Na₂SO₄, filtered and evaporated. The crude reaction product was dissolved in pentane and filtered through a pad of silica gel, the pad was further rinsed with CHCl₃. The CHCl₃ filtrate was evaporated and the resultant residue was purified via column chromatography over silica gel using a gradient of 0-2% of EtOAc/Hexanes to obtain Compound **61** (162 mg; 63%). This reaction was also performed on larger scale using 3.0 mmole of allylic halide **58** to give **61** (517 mg; 65%).

MH⁺/Z calc. for C₁₈H₂₇O₁ = 259.21; LRMS found = 259.27

¹H NMR (300 MHz, CDCl₃): δ (ppm) 1.59 (s, 3H); 1.64 (s, 3H); 1.72 (s, 3H); 1.99-2.13 (m, 4 H); 2.26-2.34 (m, 2H); 2.58-2.85 (m, 2H); 3.80 (s, 3H); 5.09-5.14 (m, 1H); 5.17-5.22 (m, 1H); 6.81-6.86 (m, 2H); 7.08-7.26 (m, 2H).

Synthesis of the racemic epoxide **63**

The racemic epoxide **63** was synthesized as described in Ceruti, M. *et al. J. Med. Chem.* **1998**, *41*, 540-554.

To a solution of Compound **61** (100 mol%; 2.0 mmole; 517 mg) in THF (58 mL) at 0°C was added water (50 mL) until the solution reached opalescence. The resultant mixture was stirred at 0°C, and *N*-bromosuccinimide (NBS; 110 mol%; 2.2 mmole; 392 mg) was added in portions over 10 min. Water was added during the addition to maintain opalescence of the solution. After the addition of NBS was completed the reaction mixture was stirred an additional 15 min at 0°C and water was continuously added to maintain opalescence (total volume of water added: 20 mL). Saturated aqueous NaHCO₃ was added (60 mL) and the resulting mixture was extracted using Et₂O (3 \times 60 mL). The organic layers were combined and washed using saturated aqueous NaHCO₃ (60 mL) and brine (60 mL). The organic

solution was dried over Na₂SO₄, filtered and evaporated. The resultant residue was purified via column chromatography over silica gel using a gradient of 0-6% EtOAc/Hexanes to provide a crude bromohydrin (487 mg; 69%).

¹H NMR (300 MHz, CDCl₃): δ (ppm) 1.34 (s, 3H); 1.34 (s, 3H); 1.54 (s, 3H); 1.76-1.84 (m, 1 H); 1.93-1.99 (m, 1H); 2.12-2.16 (m, 2H); 2.27-2.34 (m, 2H); 2.59-2.65 (m, 2H); 3.80 (s, 3H); 3.92 (dd, J₁ = 2.0 Hz, J₂ = 11 Hz, 1H); 5.24-5.29 (m, 1H); 6.80-6.85 (m, 2H); 7.08-7.26 (m, 2H).

To a suspension of K₂CO₃ (300 mol%; 3.6 mmole; 498 mg) in methanol (20 mL) was added freshly prepared crude bromohydrin, above (100 mol%; 1.2 mmole; 426 mg). The reaction mixture was stirred at room temperature (20°C) for 2.5 hrs (reaction was monitored by TLC analysis). Water was added and the resultant mixture was extracted with Et₂O (3 × 50 mL). The organic layers were combined and washed using brine (2 × 50 mL). The organic solution was dried over Na₂SO₄, filtered and evaporated. The resultant residue was purified via column chromatography over silica gel using a gradient of 0-5% EtOAc/Hexanes to provide compound **63** (261 mg; 79%).

¹H NMR (300 MHz, CDCl₃): δ (ppm) 1.28 (s, 3H); 1.32 (s, 3H); 1.59 (s, 3H); 1.61-1.72 (m, 2 H); 2.07-2.18 (m, 2H); 2.30 (dd, J₁ = 7.2 Hz, J₂ = 15 Hz, 2H); 2.60 (t, J = 7.6 Hz, 2H); 2.70 (t, J = 6.4 Hz, 1H); 3.79 (s, 3H); 5.21-5.26 (m, 1H); 6.80-6.84 (m, 2H); 7.08-7.13 (m, 2H).

Synthesis of chiral diol **62b**

The chiral diol **62b** was synthesized as described in Corey, *et al. J. Am. Chem Soc.* **1997**, *119*, 9927-9928; and Crispino, G.A. *et al. Synthesis* **1993**, 777-779.

To a mixture of **61** (100 mol%; 1.9 mmole; 500 mg) and chiral catalyst (1,4-bis(dihydroquinidinephthalazine) ((DHQD)₂PHAL) for the synthesis of the *R* enantiomer or 1,4-bis(dihydroquininephthalazine) ((DHQ)₂PHAL) for the synthesis of the *S* enantiomer (5 mol%; 96 μmol; 75.4 mg), in a 1:1 solution of water:*tert*-butanol (20 mL) at 0°C was added potassium carbonate (300 mol%; 5.8 mmole; 800 mg), potassium ferricyanide (300 mol%; 5.8 mmole; 1.90 g), methanesulfonamide (100 mol%; 1.9 mmole; 184 mg), and potassium osmate dihydrate (1 mol%, 19 μmole; 7.1 mg). An additional 15 mL of the 1:1 solution of water:*tert*-butanol was added, and the reaction mixture was stirred at 0°C for 4.5 hrs. Saturated aqueous Na₂S₂O₃ (10 mL) was added and the reaction was stirred at room

temperature (20°C) for an additional 45 min. Evaporation of the volatiles on rotary evaporator (no heat) was followed by extraction using EtOAc (4 × 30 mL). The organic layers were combined and washed with KOH (1M; 30 mL) and brine (30 mL). The organic solution was dried over Na₂SO₄, filtered and evaporated. Crude diol **62** was purified first by precipitation of tetrol (overoxydation product) in Et₂O followed by column chromatography over silica gel using a gradient of 0-40% EtOAc/Hexanes to provide Compound **62a** (184 mg; 33%) and Compound **62b** (127 mg; 22%).

¹H NMR (300 MHz, CDCl₃): δ (ppm) 1.16 (s, 3H); 1.19 (s, 3H); 1.38-1.46 (m, 1 H); 1.57 (s, 3H); 2.05-2.09 (m, 1H); 2.21-2.24 (m, 2H); 2.30 (dd, J₁ = 7.6 Hz, J₂ = 15 Hz, 2H); 2.56-2.63 (m, 2H); 3.29 (dd, J₁ = 2.0 Hz, J₂ = 11 Hz, 1H); 3.79 (s, 3H); 5.21-5.26 (m, 1H); 6.79-6.84 (m, 2H); 7.07-7.11 (m, 2H).

Synthesis of chiral epoxide **63b**

The chiral epoxides **63a** and **63b** were synthesized as described in Corey, *et al. J. Am. Chem Soc.* **1997**, *119*, 9927-9928.

The following example is for a synthesis of **63a**, but the same procedure was used for synthesis of **63b** except that chiral diol **62b** was used in place of chiral diol **62a**. To a solution of chiral diol **62a** (100 mol%; 0.60 mmole; 175 mg) in CH₂Cl₂ (4 mL) at 0°C was added pyridine (800 mol%; 4.8 mmole; 388 μL) and methanesulfonyl chloride (480 mol%; 2.8 mmole; 223 μL). The reaction mixture was stirred at 0°C for 5 min then for an additional 3 hrs at room temperature (20°C). The solvent was evaporated by nitrogen gas circulation through the reaction flask. Methanol (12 mL) was added along with potassium carbonate (1667 mol%; 10 mmole; 1.44 g), and the resultant suspension was stirred at room temperature (20°C) for 1-2 hrs (monitored by TLC analysis). Water (20 mL) was added, and the mixture was extracted using Et₂O (2 × 20 mL). The organic layers were combined and washed using dilute Cu(II) nitrate (2 × 20 mL), water (20 mL), and brine (20 mL). The organic solution was dried over MgSO₄, filtered and evaporated. Crude epoxide **63** was purified by column chromatography over silica gel using a gradient of 0-2% EtOAc/Hexanes to provide Compound **63a** (105 mg; 64% over two steps) and Compound **63b** (70 mg 64% over two steps starting from 0.40 mmole of **62b**).

¹H NMR (300 MHz, CDCl₃): δ (ppm) 1.27 (s, 3H); 1.31 (s, 3H); 1.58 (s, 3H); 1.60-1.72 (m, 2H); 2.09-2.28 (m, 2H); 2.30-2.33 (m, 2H); 2.57-2.62 (m, 2H); 2.70 (t, J = 6.4 Hz, 1H); 3.79 (s, 3H); 5.20-5.25 (m, 1H); 6.79-6.84 (m, 2H); 7.07-7.12 (m, 2H).

Synthesis of Compound 64b

Compounds **64a** and **64b** were synthesized as described in Corey, *et al. J. Am. Chem Soc.* **1997**, *119*, 9927-9928.

The following example is for synthesis of Compound **64a**, but the same procedure was used for synthesis of Compound **64b** except that chiral epoxide **63b** was used in place of chiral epoxide **63a**. To a solution of chiral epoxide **63a** (100 mol%; 0.38 mmole; 105 mg) in dichloromethane (3 mL) at -78°C was added a solution of methylaluminum dichloride (120 mol%; 0.46 mmole; 460 µL of a 1M solution in hexanes) in dichloromethane (3 mL) cooled at -78°C via cannula. The reaction mixture was stirred at -78°C for 4 hrs. Triethylamine (0.80 mL) and 1:1 water:methanol (0.80 mL) were added, the resultant mixture was poured onto half saturated aqueous ammonium chloride (20 mL) and extracted using dichloromethane (10 mL) and Et₂O (2 × 20 mL). The organic layers were combined and washed using brine (30 mL). The organic solution was dried over Na₂SO₄, filtered and evaporated. The resultant residue was purified via column chromatography over silica gel using a gradient of 0-4% EtOAc/Hexanes to provide Compound **64a** (52 mg; 50%) and **64b** (38 mg, 56% starting from 0.25 mmole of **63b**).

¹H NMR (300 MHz, CDCl₃): δ (ppm) 0.91 (s, 3H); 1.09 (s, 3H); 1.22 (s, 3H); 1.25-1.30 (m, 1H); 1.44-1.62 (m, 1H); 1.67-1.94 (m, 4H); 2.25-2.32 (m, 1H); 2.74-2.96 (m, 2H); 3.29-3.34 (m, 1H); 3.78 (s, 3H); 6.68-6.69 (m, 1H); 6.78-6.79 (m, 1H); 6.95-6.98 (m, 1H).

Synthesis of Compound 65b

Compounds **65a** and **65b** were synthesized as described in Ley, S.V. *et al.*; Marsden S.P. *Synthesis* **1994**, 639-666.

The following example is for a synthesis of Compound **65a**, but the same procedure was used for synthesis of Compound **65b** except that Compound **64b** was used in place of Compound **64a**. To 4Å dry powdered molecular sieves (121 mg) was added a solution of Compound **64a** (100 mol%; 0.19 mmole; 52 mg) in dichloromethane (3 mL), 4-methylmorpholine *N*-oxide (200 mol%; 0.38 mmole; 45 mg), and tetrapropylammonium perruthenate (10 mol%; 19 µmole; 7.0 mg). The reaction mixture was stirred at room temperature (20°C) for 1-2 hrs (monitored by TLC analysis). The reaction mixture was filtered through a pad of silica gel, which was rinsed using 5% EtOAc/Hexanes (40 mL). The filtrate was discarded, and the silica gel was washed with 100% EtOAc (40 mL). The filtrate

was evaporated to provide Compound **65a** (52 mg; 99%) and Compound **65b** (36 mg; 96% starting from 0.14 mmole of **64b**).

¹H NMR (300 MHz, CDCl₃): δ (ppm) 1.15 (s, 3H); 1.18 (s, 3H); 1.31 (s, 3H); 1.77-1.84 (m, 2H); 1.89-2.10 (m, 2H); 2.41-2.50 (m, 1H); 2.60-2.91 (m, 4H); 3.78 (s, 3H); 6.68-6.71 (m, 1H); 6.78-6.79 (m, 1H); 6.98-7.00 (m, 1H).

Enantiomeric excess of Compound **65a** and Compound **65b** was for Compound **65a** (*R* enantiomer) = 21.57 min 97% ee. Retention time (Rt) for Compound **65b** (*S* enantiomer) = 21.57 min 92% ee.

Synthesis Compound **56b**

Compounds **56a** and **56b** were synthesized as described in Poigny, S. *et al.*, *J. Org. Chem.* **1998**, *63*, 5890-5894; and Ito, Y. *et al.* *J. Org. Chem.* **1978**, *43*, 1011-1013.

The following example is for a synthesis of Compound **56a**, but the same procedure was used for synthesis of Compound **56b** except that Compound **65b** was used in place of Compound **65a**. To a solution of Compound **65a** (100 mol%; 0.23 mmole; 64 mg) in dichloromethane (4 mL) at 0°C was added trimethylsilyl trifluoromethanesulfonate (200 mol%; 0.46 mmole; 84 μL), and the resulting mixture was stirred at 0°C for 5 min. Triethylamine (250 mol%; 0.58 mmole; 81 μL) was added, and the reaction mixture was stirred at 0°C for 1 hr (monitored by TLC analysis). Saturated aqueous NaHCO₃ was added (10 mL,) and the resultant mixture was extracted using Et₂O (3 × 10 mL). The organic layers were combined and washed using brine (10 mL). The organic solution was dried over MgSO₄, filtered and evaporated. Conversion of ketone **65a** to its corresponding silyl enol ether appeared to be quantitative, and the silyl enol ether was used without further purification in the next step.

¹H NMR (300 MHz, CDCl₃): δ (ppm) 0.00 (s, 9H); 0.75 (s, 3H); 0.87 (s, 3H); 1.03 (s, 3H); 1.41-1.45 (m, 2H); 1.56-1.61 (m, 1H); 1.92-1.98 (m, 1H); 2.21-2.29 (m, 1H); 2.51-2.67 (m, 2H); 3.54 (s, 3H); 4.51-4.54 (m, 1H); 6.42-6.45 (m, 1H); 6.56-6.57 (m, 1H); 6.71-6.74 (m, 1H).

To a solution of the silyl enol ether above (100 mol% 0.23 mmole; 80 mg) in acetonitrile (4 mL) was added palladium (II) acetate (200 mol%; 0.47 mmole; 105 mg). The resultant reaction mixture was stirred at reflux for 1 hr. Palladium (II) acetate (100 mol%; 0.23 mmole; 105 mg) was added, and the reaction mixture was stirred at reflux for 30 min.

The reaction mixture was cooled to room temperature (20°C) and stirred for 11.5 hrs, then stirred at reflux for 1 hr. The reaction mixture was then cooled to room temperature (20°C), filtered and evaporated. Crude enone **56a** was purified via column chromatography over silica gel using a gradient of 0-4% EtOAc/Hexanes to provide Compound **56a** (40 mg; 63%) and Compound **56b** (40 mg; 65% starting from 0.23 mmole of **65b**).

Synthesis of Compound 50a and Compound 50b

Compound **50a** and Compound **50b** were made from Compound **56a** and **56b**, respectively, according to the procedure, above, used to make Compound **50** from Compound **56**. Enantiomeric excess of Compound **50a** and Compound **50b** was measured using HPLC analysis with a chiral column (Chiralcel OD-RH, 4.6 × 150 mm): mobile phase A = 100 mM KPF₆ in water (pH2), mobile phase B = 100% acetonitrile, isocratic 40% acetonitrile 60% water at 0.5 mL/min over 60 min, column temperature 30°C, on a Waters Alliance HPLC System 2795 using a PDA detector (200 to 600 nm). Retention time (Rt) for Compound **50a** (R enantiomer)=67.25 min 95% ee. Retention time (Rt) for Compound **50b** (S enantiomer)=70.92 min 93% ee.

Synthesis of Compound 66, 67, 68 and 69

Compounds **66**, **67**, **68** and **69** were synthesized according to the method used to synthesize Compound **50**, except:

for Compound **66**, ethyl iodide was used instead of iodomethane;

for Compound **67**, propenyl iodide was used instead of iodomethane;

for Compound **68**, benzyl iodide was used instead of iodomethane; and

for Compound **69**, CH₃CH₂C(O)C(=CH₂)C(O)OCH₃ was used instead of ethyl vinyl ketone.

Synthesis of Compound 70

Compound **70** was synthesized according to the method used to synthesize Compound **50**, except that twice as much chromium(VI) oxide was used and that the corresponding oxidation was performed at 80°C.

6.4 **COMPOUND 48 SELECTIVELY INDUCES APOPTOSIS**

The ability of Compound 48 to selectively induce apoptosis in a variety of cancer cells is described below.

A wide range of anticancer agents, including chemotherapeutic agents, induces apoptosis in malignant cells *in vitro*. Without being bound by theory, apoptosis is a regulated process manifested by the activation of proteolytic cleavages resulting mostly from the action of a unique family of cysteine- proteases called caspases. To demonstrate the ability of Compound 48 to trigger caspase activation, lysates of cells treated with various concentrations of the Compound 48 were prepared. In particular, H1299 non-small cell lung carcinoma cells, C33A cervical carcinoma cells, Mrc-5 normal lung fibroblasts (American Type Culture Collection, Manassas, VA USA) and HMEC normal mammary epithelial cells (Clonetics San Diego, CA, USA) were maintained in RPMI 1640 media supplemented with 10% fetal calf serum (Sigma-Aldrich Inc., St. Louis, Missouri USA). Cells were harvested and suspended at 0.6×10^6 cells/mL in media. A 45 μ L aliquot of cell-suspension was added to each well of a 96-well microtiter plate (PerkinElmer Life Sciences Inc, Boston, MA USA). Cells were incubated overnight in a 5% CO₂, 95% humidity incubator at 37 °C and then, 5 μ L of a 10% dimethyl sulfoxide (Sigma-Aldrich Inc., St. Louis, Missouri USA) solution containing various concentrations of Compound 48 or 5 μ L of 10% dimethyl sulfoxide (solvent control) was added. The plates were further incubated for 16 hr. Cells were lysed in lysis buffer (50mM Hepes pH 7.4; 0.1% Chaps; 10 mM EDTA; 10mM DTT) and set aside for the caspase activity assay.

To demonstrate the caspase activity in the cell lysates, 0.35 μ g of N-terminal biotinylated EGKRKGDEVDGVPDRRASV peptide (Phoenix Pharmaceuticals Inc, Belmont, CA USA) was labeled with 1 mCi of ³²P- γ ATP (PerkinElmer Life Sciences Inc, Boston, MA USA) using 250 units of Protein Kinase A catalytic subunit from bovine heart (Sigma-Aldrich Inc., St. Louis, Missouri USA) in 500 μ L of HMK buffer (20 mM pH 7.5 Tris-HCl; 0.1 M NaCl; 12 mM MgCl₂; 1mM DTT) at 37°C for one hour. The reaction was then filtered using Sephadex G-10 Poly-Prep chromatography column (Amersham Biosciences, Inc, Piscataway, NJ, USA). The labeled peptide was coupled to 1.25 mL of streptavidin sepharose beads (Amersham Biosciences, Inc, Piscataway, NJ, USA) during 15 minutes at room temperature on a rotary mixer. The beads were washed seven times with 6 mL of 0.5M NaCl in PBS and resuspended in a total volume of 7.25 mL of 0.5 M NaCl PBS solution to which 9

mL of RPMI 1640 media was added. 96-well 0.45 μm MultiScreen-HV filter plates (Millipore, Bedford, Mass USA) were then prewetted with 200 μL of 0.5M NaCl in PBS and 40 μL of beads suspension was added to each well. Each well was washed five times with 200 μL of 0.5 M NaCl in PBS. In each well, 50 μL of cell lysate was added together with 12.5 μL of 0.5 M NaCl in 30% glycerol solution to each well. The plates were incubated at 30°C with shaking at 220 rpm overnight. On the next day, the filter plates containing the beads and the extract were placed on top of 96-well sample plates (PerkinElmer Life Sciences Inc, Boston, MA USA) containing 100 μL of Optiphase SuperMix liquid scintillant fluid (PerkinElmer Life Sciences Inc, Boston, MA USA) in each well and centrifuged at 1500 rpm for 10 minutes at room temperature. The number of radioactive counts per minute (cpm) in each well of the sample plate was measured using a liquid scintillation counter (PerkinElmer Life Sciences Inc, Boston, MA USA). The potency of caspase cascade activation was determined by the percentage increase in cpm in wells compared to cells treated with dimethyl sulfoxide only. Values two fold higher (200%) than control were considered positive and demonstrated that the compound triggered caspase activation in the cells.

As depicted in Figure 2, results showed that caspases were activated in the cancer cell lines H1299 and C33A and not in the normal cell line following 16 hours incubation with 1.6 μM of Compound 48. These results demonstrate that Compound 48 induces apoptosis selectively in cancer cells and is useful for treating or preventing cancer, particularly lung or cervical cancer.

6.5 COMPOUND 48 SELECTIVELY AFFECTS CANCER-CELL VIABILITY

To demonstrate the effect of Compound 48 on cell viability, cellular ATP levels were measured following Compound 48 treatment. H1299 non-small cell lung carcinoma cells, C33A cervical carcinoma cells, Mrc-5 normal lung fibroblasts (American Type Culture Collection, Manassas, VA USA) and HMEC normal mammary epithelial cells (Clonetics San Diego, CA, USA) were cultured in RPMI 1640 media supplemented with 10% fetal calf serum (Sigma-Aldrich Inc., St. Louis, Missouri USA). The four cells lines were plated in 96-well microtiter plates (PerkinElmer Life Sciences Inc, Boston, MA USA) at a confluency that allowed them to reach confluence after 4 days of growth. One day after plating, the cells were treated either with 10 μM etoposide, 100 nM staurosporine or 1.6 μM of Compound 48. Stock solutions of each compound were prepared in dimethyl sulfoxide (Sigma-Aldrich Inc., St. Louis, Missouri USA), diluted in media then added to the cells. The total dimethyl

sulfoxide on the cells was 1%. After 3 days of incubation, the ATP levels in the cells were quantified using the luminescent ViaLight detection system (Bio-Whittaker, MD, USA). The results were plotted relative to untreated control cells, which were set at 100.

As depicted in Figure 3, Compound **48** has a significantly greater effect on the ATP levels in cancer cells than in normal cells. After 72 hours, treatment with 1.6 μ M Compound **48** was significantly more effective at lowering ATP levels in cells of the cancer cell line H1299 and C33A compared with cells of the lines HMEC and MRC-5. Control compounds were tested to illustrate the significance of this effect. The anti-cancer drug etoposide as well as Compound **48**, but not the protein kinase inhibitor staurosporine, are selectively cytotoxic towards cancer cells, particularly lung and cervical cancer. These results demonstrate that Compound **48** is selectively cytotoxic to cancer cells and is useful for treating or preventing cancer, particularly lung or cervical cancer.

6.6 ANTI-ONCOGENIC EFFECTS OF COMPOUND 50

6.6.1 EFFECTS OF COMPOUND 50A AND COMPOUND 50B ON CANCER CELL VIABILITY *IN VITRO*

To demonstrate the effect of Compounds **50a** and **50b**, respectively, on cell viability, cellular ATP levels were measured before and after treating cell lines with each compound. The following cell lines were used: A549 human non-small cell lung carcinoma; C33A human cervical carcinoma; H1299 human non-small cell lung carcinoma; Hep-3B human hepatocellular carcinoma; MIA-Paca-2 human pancreatic carcinoma; PC-3 human prostatic adenocarcinoma (American Type Culture Collection, Manassas, VA, USA); HMEC normal human mammary epithelial cells; HUVEC normal human umbilical vein endothelial cells; and NHBE normal human bronchial epithelial cells (Cambrex Bio Science, Rockland, ME, USA). All tumor cell lines were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, VT, USA), 100 U/mL penicillin, 100 μ g/mL streptomycin and 2 mM L-glutamine (Invitrogen, Carlsbad, CA, USA). HMEC, HUVEC and NHBE cell lines were cultured in MEGM, EGM-2 and BEGM media, respectively (Cambrex Bio Science, Rockland, ME, USA). The cell lines were plated in 96-well microtitre plate at a density of $2-4 \times 10^3$ cells per well. After 16 hours, the cells were treated with various concentrations of Compound **50a** or Compound **50b**. Stock solutions of Compound **50a** and Compound **50b**, respectively, were prepared in dimethyl sulfoxide at a concentration of 5 mM. Serial dilutions were prepared in RPMI, 10% FBS and 2% DMSO and then added to

the cells. The total DMSO in the cell cultures was 1%. After 72 hours of incubation, the ATP levels in the cells were quantified using the Vialight HS kit (Cambrex Bio Science, Rockland, ME, USA). The results were plotted relative to untreated control cells, which were set at a value of 100. The IC50s were determined using a best-fit sigmoidal dose response curve with variable slope.

As depicted in Table 5, Compound **50a** and Compound **50b** showed greater efficacy in decreasing cellular ATP levels in the cancer cell lines than in normal cell lines. These results demonstrate that Compound **50a** and Compound **50b** are selectively cytotoxic to cancer cells and are useful for treating or preventing cancer, particularly NSCL-LC carcinoma, NSCL-adrenocarcinoma, liver cancer, pancreatic cancer, cervical cancer, prostate cancer or lung cancer.

Table 5. Anti-oncogenic effects of Compound **50a** and Compound **50b**

	Cell line	Tissue type	Species	IC50 of Compound 50a (μ M)	IC50 of Compound 50b (μ M)
Tumor	H1299	NSCL-LC carcinoma	Human	0.550	0.230
	A549	NSCL- adrenocarcinoma	Human	0.630	0.580
	Hep 3B	Liver	Human	0.970	0.770
	MIA- Paca-2	Pancreas	Human	0.660	0.250
	C33A	Cervix	Human	0.500	0.450
	PC-3	Prostate	Human	0.370	0.350
Normal	HMEC	Breast epithelial	Human	2.300	2.100
	HUVEC	Endothelial	Human	1.700	1.300
	NHBE	Bronchial epithelial	Human	1.200	1.200

* Measurement of ATP levels were taken 72 h post-treatment and compared to untreated cells.

6.6.2 EFFECT OF COMPOUND 50 ON GROWTH OF CERVICAL TUMOR CELLS *IN VIVO*

To demonstrate the anti-tumor activity of Compounds **50** *in vivo*, CB17 SCID/SCUD female mice (Charles River, MA, USA) that were injected with C33A human cervical cancer cells were used. The resultant mice are a model for a human having cervical cancer.

The C33A human cervical cancer cells were maintained in RPMI supplemented with 10% inactivated fetal bovine serum and 1% penicillin-streptomycin-L-Glutamine, under 5% CO₂ at 37°C, and passaged twice a week. The cells were grown at a confluency lower than 70% and then collected with Trypsin (Bio-Whittaker, MD, USA). The cells were then centrifuged and washed twice using phosphate buffered saline solution (PBS) and resuspended in PBS at 2 X 10⁶ cells per 100 µl. Viability was examined by staining with Trypan Blue and only flasks with cell viability of greater than 95% were used for *in vivo* studies.

C33A cells were transplanted subcutaneously into the flank of female CB17 SCID/SCID mice. Each mouse was inoculated with a suspension of 2 X 10⁶ tumor cells per 100 µL on day zero. The following six treatment groups of ten mice each were used: (a) a negative control group treated with intravenous (i.v.) injection of vehicle, (b) a negative control group treated with intraperitoneal (i.p.) injection of vehicle, (c) a positive control group treated with i.p. injection of Cisplatin, (d) a group treated with i.v. injection of a Compound **50**, (e) a group treated with i.p. injection of Compound **50**, and (f) a group treated with subcutaneous (s.c.) injection of Compound **50**.

Treatment started on day thirteen after C33A cells transplantation for Compound **50** treated groups and on day fourteen for Cisplatin treated group. Compound **50** was administered i.v., i.p., and s.c. once daily for five consecutive days at a dose of 10, 15 and 30 mg/kg, respectively. Compound **50** was prepared as a working solution of 1.5 mg/mL in a vehicle solution of 20% Cremaphor EL (Sigma, St. Louis, MS, USA), 10% Ethanol and 5% Dextrose (Abbot Laboratories, QC, Canada). The negative control groups were treated with vehicle alone. The positive control group was treated once every 3 days for fifteen days at a dose of 3.5 mg/kg. Cisplatin was formulated in PBS on each day of the injection and was administered i.p.

The mice were weighed and the tumors measured on day 13 and every 2 to 3 days after treatment commenced. Observations continued for 36 days after initial tumor implantation. The changes in body weight and in the calculated tumor volume were plotted

(Figures 4 and 5). Statistical analysis was performed using GraphPad Prism (GraphPad Software Inc., San Diego, CA). Two-way ANOVA was used to determine how the treatment affected tumor growth over time. Following the two-way ANOVA, post-tests were performed using the Benferroni method to determine the statistical difference between the mean tumor-size of the two groups being compared on every day that the tumors were measured. Four animals in the negative control injected i.v. with vehicle and two animals in the group treated i.v. with Compound **50** died during the observation period. Therefore, the data collected from these animals were excluded from all calculations and the total number of animals in the groups changed to six for the negative control injected i.v. with vehicle and eight for the group treated i.v. with Compound **50**.

As shown in Figure 4, mice treated with Compound **50** experienced a non-significant change in body weight, whereas Cisplatin treated positive control group experienced a weight loss of 19% on day 34.

As shown in Figure 5, Compound **50** administered i.v. or i.p. at a dose of 10 or 15mg/kg, respectively, once a day for five days resulted in a significant reduction ($p<0.001$) in tumor growth compared to mice treated i.v. with vehicle only. On day 36, animals treated i.v. with 10 mg/kg of Compound **50** had significantly ($p<0.05$) smaller mean tumor-size than animals treated with vehicle only. Similarly, animals treated i.p. with Compound **50** had a significantly ($p<0.05$) smaller mean tumor-size than animals treated with vehicle on day 34 and day 36. Furthermore, Compound **50** administered s.c. at a dose of 30 mg/kg once a day for five days resulted in a significant reduction ($p<0.001$) in tumor growth compared to mice treated s.c. with vehicle alone. Animals treated s.c. with Compound **50** had a significantly smaller mean tumor-size than animals treated with vehicle on day 34 ($p<0.01$) and day 36 ($p<0.001$).

As indicated in Figure 5, Compound **50** significantly reduces the human cervical tumors implanted in SCID mice, an art-accepted model for human cervical cancer. Accordingly, Compound **50** is useful for inhibiting the growth of a cancer cell, particularly a cervical cancer cell, and for treating or or preventing cancer, particularly cervical cancer, in a patient.

6.7 ANTI-FUNGAL BIOLOGICAL ACTIVITY OF COMPOUND 50

6.7.1 EFFECT OF COMPOUND 50 ON THE GROWTH OF THE YEAST *SACCHAROMYCES CEREVISIAE*

To demonstrate that Compound **50** inhibits the growth of the yeast *Saccharomyces cerevisiae*, the strain W303a was cultured in YEP media, containing Bacto Yeast Extract and Bacto Peptone (Becton Dickinson Microbiology Systems, Sparks, MD, USA) and supplemented with one of three different carbon sources, 2% glucose (Sigma Chemical Co. St. Louis, MO, USA), 2% galactose (BDH Laboratory Supplies, Poole, England), or 2% glycerol (EM Science, Gibbstown, NJ, USA). Yeast cells were first cultured overnight in 10 mL of YEP glucose-containing medium at 30°C. The following day, 5 µL of the overnight culture ($5-6 \times 10^3$ cells) was added to 100 µL of YEP media supplemented with 2% of one of the three carbon sources mentioned above in a 96-well microtitre plate. To these cultures a dilution series of Compound **50** was added. Compound **50** was prepared as a stock solution in 100% dimethyl sulfoxide (DMSO) at a concentration of 10 mM. Serial dilutions were prepared in distilled water. The final DMSO concentration in each well was 0.5%. Yeast cells were grown at 30°C and cell growth was monitored by reading the OD₆₀₀ (optical density at 600 nm) with a Tecan ULTRA plate reader (TECAN U.S. Inc., Research Triangle Park, NC, USA). As depicted in Figure 6, Compound **50** significantly inhibited growth of the yeast *Saccharomyces cerevisiae* in dose-dependent manner. Accordingly, Compound **50**, an illustrative Diterpenoid Compound, is useful for inhibiting the growth of a fungus or treating a fungal infection.

6.7.2 EFFECT OF COMPOUND 50 ON GROWTH OF THE FUNGUS *CANDIDA ALBICANS*

To demonstrate that Compound **50** inhibits the growth of the fungus *Candida albicans*, the strain ATCC 10231 was cultured in YEP media, containing Bacto Yeast Extract and Bacto Peptone (Becton Dickinson Microbiology Systems, Sparks, MD, USA) and supplemented with 2% glucose (Sigma Chemical Co. St. Louis, MO, USA). Fungus cells were first cultured overnight in 10 mL of YEP glucose-containing medium at 30°C. The following day, 5 µL of the overnight culture ($5-6 \times 10^3$ cells) was added to 100 µL of YEP glucose-containing medium in a 96-well microtitre plate. To these cultures a dilution series of Compound **50** was added. Compound **50** was prepared as a stock solution in 100%

dimethyl sulfoxide (DMSO) at a concentration of 10 mM. Serial dilutions were prepared in distilled water. The final DMSO concentration in each well was 0.5%. Fungus cells were grown at 30°C and cell growth was monitored by reading the OD₆₀₀ (optical density at 600 nm) with a Tecan Ultra plate reader (TECAN U.S. Inc. Research Triangle Park, NC, USA). As depicted in Figure 7, Compound **50** significantly inhibited growth of the fungus *Candida albicans* in a dose-dependent manner. Accordingly, Compound **50**, an illustrative Diterpenoid Compound, is useful for inhibiting the growth of a fungus or treating a fungal infection.

6.8 BIOLOGICAL ACTIVITY OF COMPOUNDS 66, 67, 68, 69 AND 70

6.8.1 EFFECTS OF COMPOUNDS 66, 67, 68, 69 AND 70 ON CANCER CELL VIABILITY *IN VITRO*

To demonstrate the effect of compounds **66, 67, 68, 69** and **70** on cell viability, cellular ATP levels were measured before and after treating cell lines with each compound. The selected tumor cell lines included C33A human cervical carcinoma and H1299 human non-small cell lung carcinoma (American Type Culture Collection, Manassas, VA, USA). The cell lines were cultured in RPMI 1640 media supplemented with 10% FBS (Hyclone, Logan, VT, USA), 100 U/mL penicillin, 100 µg/mL streptomycin and 2 mM L-glutamine (Invitrogen, Carlsbad, CA, USA). The cell lines were plated in 96-well microtitre plate at a density of $2-4 \times 10^3$ cells per well. After 16 hours, the cells were treated with various concentrations of compound **66, 67, 68, 69** and **70**, respectively. Stock solutions of compounds **66, 67, 68, 69** and **70** were prepared in dimethyl sulfoxide at a concentration of 5 mM. Serial dilutions were prepared in RPMI, 10% FBS and 2% DMSO and then added to the cells. The total DMSO on the cells was 1%. After 72 hours of incubation the ATP levels in the cells were quantified using Vialight HS kit (Cambrex Bio Science, Rockland, ME, USA). The results were plotted relative to untreated control cells, which were set at a value of 100. The IC₅₀s were determined using a best-fit sigmoidal dose response curve with variable slope.

As depicted in Table 6, treatment with Compound **66, 67, 68, 69** or **70** decreased the cellular ATP levels in the cancer cell lines. These results demonstrate that Compounds **66, 67, 68, 69** and **70** inhibit the growth of a cancer cell, particularly a C33A human cervical carcinoma cell and H1299 human non-small cell lung carcinoma cell, and are useful for

treating or preventing cancer, particularly human cervical carcinoma and human non-small cell lung carcinoma, in a patient.

Table 6. The IC₅₀s in μ M of Compounds **66**, **67**, **68**, **69** and **70** for anti-oncogenic effects*.

Compound	Formula	H1299	C33A
66	I	1.440	1.550
67	I	0.950	1.190
68	I	2.070	2.530
69	I	1.360	0.870
70	III	16.810	15.890

* Measurement of ATP levels were taken 72 h post-treatment and compared to untreated cells.

The present invention is not to be limited in scope by the specific embodiments disclosed in the examples which are intended as illustrations of a few aspects of the invention and any embodiments that are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art and are intended to fall within the scope of the appended claims.

A number of references have been cited, the entire disclosures of which have been incorporated herein in their entirety.